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Central administration of neuropeptide Y induces wakefulness in rats

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Szentirmai, E. and J. M. Krueger. Central administration of neuropeptide Y induces wakefulness in rats. Am J Physiol Regul Integr Comp Physiol 291: R473–R480, 2006. First published February 23, 2006; doi:10.1152/ajpregu.00919.2005.—Neuropeptide Y (NPY) is a well-characterized neuromodulator in the central nervous system, primarily implicated in the regulation of feeding, NPY, orexins, and ghrelin form a hypothalamic food intake regulatory circuit. Orexin and ghrelin are also implicated in sleep-wake regulation. In the present experiments, we studied the sleep-modulating effects of central administration of NPY in rats. Rats received intracerebroventricular injection of physiological saline or three different doses of NPY (0.4, 2, and 10 μg in a volume of 4 μl) at light onset. Another group of rats received bilateral microinjection of saline or 2 μg NPY in the lateral hypothalamus in a volume of 0.2 μl. Sleep-wake activity and motor activity were recorded for 23 h. Food intake after the control and treatment injections was also measured on separate days. Intracerebroventricular and lateral hypothalamic administration of NPY suppressed non-rapid-eye-movement sleep and rapid-eye-movement sleep in rats during the first hour after the injection and also induced changes in electroencephalogram delta power spectra. NPY stimulated food intake in the first hour after both routes of administration. Data are consistent with the hypothesis that NPY has a role in the integration of feeding, metabolism, and sleep regulation.

NPY is widely distributed in high concentrations in the central nervous system and acts as a neurohormone and neuromodulator. The main source of NPY in the brain is the hypothalamus, particularly the arcuate nucleus (ARC), dorsomedial nucleus (DMN), paraventricular nucleus (PVN), suprachiasmatic nucleus (SCN), and the brain stem (2). NPY is implicated in the regulation of several physiological processes, such as food intake (24, 38), hormone secretions (13, 23, 42), circadian rhythms (1), thermoregulation (19), and blood pressure (7). NPY is part of the widely studied hypothalamic food intake regulatory circuit that also involves orexin, ghrelin, agouti-related peptide, and melanin-concentrating hormone. All of these neuropeptides stimulate food intake when injected in the cerebral ventricle or in various hypothalamic nuclei (9, 20, 34–36, 46). The NPY receptor family includes at least six subtypes from which the Y1 and Y5 are implicated in the regulation of food intake. Both receptors are present in the PVN, ARC, medial preoptic area, SCN, supraoptic nucleus, and in the lateral hypothalamus (LH; see Ref. 44). The NPY Y1 receptor may be involved in mediating behavioral effects other than feeding, since mice lacking NPY Y1 receptor show reduced locomotor activity (33).

NPY-immunoreactive neurons, originating from the ARC, innervate orexigenic cells in the LH (16). Orexin-immunoreactive axon terminals from the LH end on NPYergic neurons in the ARC (16, 28). Ghrelin is known to act through NPYergic pathways in the ARC (22, 37) to stimulate feeding. Orexin is known to play an important role in maintaining wakefulness (36). Orexin stimulates wakefulness when injected in the cerebral ventricles (14) or in the PVN, DMH, and LH (8). The lack of orexin and/or orexin receptors is linked to narcolepsy (26). Ghrelin also inhibits sleep in rats when injected in the cerebral ventricle (41) or various hypothalamic nuclei (unpublished observations).

Little is known concerning the potential role of NPY in sleep regulation. In one study, visual inspection of the electroencephalograms (EEG) suggested that NPY induces a reduction in desynchronized EEG activity and an increase in synchronized and mixed activity in rats (49). Ehlers at al. (11) found that high doses of NPY decrease EEG power of all frequencies in rats but does not influence sleep onset or the amount of non-rapid-eye-movement sleep (NREMS). In humans, repeated intravenous injection of NPY was reported to promote sleep and reduce sleep latency when given to young normal male subjects (3). The same research group in a more recent study that was done in older male and female patients with depression using age-matched controls found no change in sleep time, only shortened sleep latency after repeated intravenous administration of NPY (15). The aim of our experiments was to study sleep and EEG responses to centrally injected NPY in rats.

METHODS

Animals. Male Sprague-Dawley rats, weighing 275–300 g at the time of surgeries, were used. Rats were housed individually in Plexiglas cages in temperature-controlled (23 ± 1°C) environmental chambers at a 12:12-h light-dark cycle (light on at 9:00 A.M.). Water and food were available ad libitum. Institutional guidelines for the care and use of research animals were followed and approved by the Institutional Animal Care and Use Committees.

Surgery. Rats were anesthetized by intraperitoneal injection of a ketamine and xylazine mixture (87 and 13 mg/kg, respectively). Stainless steel screw electrodes for EEG recordings were placed over the frontal (1.5 mm anterior and 1.5 mm lateral to the bregma) and parietal (4 mm posterior and 2 mm lateral to the bregma) cortices, and electromyographic (EMG) electrodes were implanted in the dorsal neck muscle. Stereotaxic equipment was used to insert an intracerebroventricular cannula (22-gauge; Plastics One) in the right lateral cerebral ventricle [coordinates of the tip of the guide cannula: 0.80

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mm posterior and 1.5 mm lateral to the bregma, and 4.0 mm ventral from the surface of the skull, according to the rat brain atlas by Paxinos and Watson (32) or microinjection cannulas (26 G) bilaterally in the LH (coordinates of the tip of the guide cannula: 2.1 mm posterior, 2 mm lateral, and 7.8 mm ventral). The size of the injector cannulas were 30 gauge for the intracerebroventricular cannula and 33 gauge for the microinjection cannulas: both extended 0.5 mm beyond the tip of the guide. The guide cannulas and the screws were fixed with dental cement to the skull.

Verification of cannula placement. The location of the intracerebroventricular cannula was determined by the gravity method (sudden drop in pressure) during implantation, and the drinking response to intracerebroventricular injection of angiotensin (Bachem, Torrance, CA) was tested 3–4 days after surgery and also after the end of the experiments. To verify the location of the microinjection cannulas in the LH, 0.2 µl of 5% horseradish peroxidase was injected in the cannulas at the end of the experiment. Rats, anesthetized with isoflurane were perfused with saline and 4% paraformaldehyde. Brains were removed and kept at 4°C in paraformaldehyde until further examinations. The peroxidase-H2O2 reaction was visualized by diaminobenzidine in 100-µm-thick neutral red-stained coronal brain sections. The spread of the injections was <1 mm, as indicated by the enzyme reaction. The injection sites were localized with reference to the rat brain atlas (32 and Fig. 1).

Sleep-wake recording. After surgery, a 7- to 10-day recovery period followed, and then the rats were connected to the recording cable and habituated to the experimental conditions for an additional 5 days. The recording cables were attached to commutators. Cables from the commutators were connected to amplifiers. The digitized (128-Hz sampling rate) signals of the EEG and EMG were collected by computers. The EEG was filtered <0.1 Hz and >40 Hz. EMG activity served the purpose of aiding in determining the vigilance state of the animals. The states of vigilance were determined off-line in 10-s epochs by using the conventional criteria as NREMS [high-amplitude EEG waves, lack of body movement, predominant EEG power in the delta range (0.5–4.0 Hz)]; rapid-eye-movement sleep (REMS; highly regular low-amplitude EEG, dominance of theta activity with corresponding high-fast-Fourier transformation (FFT) theta (4.5–8 Hz) power, general lack of body movements with occasional twitches]; and wakefulness (less regular low-amplitude EEG, the lack of the visible theta dominance, and frequent body movements). The amount of time spent in each vigilance state was calculated in 1-h time blocks. Power density values were calculated for each vigilance state by FFT for consecutive 10-s epochs in the frequency range of 0.5–16.0 Hz for 0.5-Hz bands. In addition, EEG power values for the 0.5- to 4-Hz delta range during NREMS were integrated and used to characterize NREMS intensity, also known as EEG slow-wave activity (SWA). Those epochs that contained EEG artifacts were excluded from the FFT analyses.

Experimental procedures. In experiment 1, rats were injected intracerebroventricularly with NPY (Bachem California) or pyrogen-free isotonic NaCl (PFS) 10–15 min before light onset. The three doses of NPY were of 0.4 µg (n = 8), 2 µg (n = 9), and 10 µg (n = 8) injected in a volume of 4 µl. In each group, two conditions, a baseline day when 4 µl of PFS was administered and an experimental day when NPY was injected, were used. The order of the baseline and experimental days was chosen randomly. Some of the rats were injected with more than one dose of NPY; at least 1 wk separated the injections. These rats were not selected based on previous responses to NPY. In experiment 2, another group of rats (n = 8) with bilateral intrahypothalamic cannulas received 2 µg NPY/injection on each side in a volume of 0.2 µl on the experimental day and equal volumes of PFS on the control day. Microinjections took place over a 1-min period, and the microinjection cannulas were left in place for one additional minute. The rats were adapted to the experimental procedures for at least 7 days before the experiments; by the time of the recording, the injection procedures did not cause any visible stress or discomfort to the rats. Each rat was recorded beginning at 9:00 A.M. for 23 h, starting immediately after injections.

Measurement of food intake. Food intake was measured in each group of rats 4 days after the sleep studies. The experimental procedures were similar to those above. Immediately after injection, animals were returned to their home cages where a known amount of chow had been placed. Food pellets were reweighed 1 h after injection. Results are expressed as gram food intake per kilogram body weight ± SE.

Statistics. Two-way ANOVA for repeated measures was performed on sleep and power spectra data (factors: treatment and time effect or treatment and frequency effect, respectively). Those hours, during which a rat did not have at least 5 min NREMS, were excluded from the SWA analysis, resulting in missing data points. Therefore, instead of repeated-measures ANOVA, two-way ANOVA was performed on SWA. Time spent in sleep and SWA data were analyzed in 1-h time blocks for hours 1, 2, and 3 and on 3-h time blocks for hours 4–23 of the recording period between the baseline day and the experimental days in each group. Average power spectra values during each vigilance state were analyzed in the first 3 h after injections. When ANOVA indicated significant effects, the Student-Newman-Keuls test (SNK test) was used for post hoc analysis to identify which group and treatment differed from the other groups and treatments. The episode numbers, the average episode duration of NREMS and REMS, and the effects of NPY on food intake in the first hour after the injection were analyzed by paired t-test. When at least half of the rats did not have a REMS episode in that hour, statistical analysis on average REMS episode duration was not performed. An α-level of P < 0.05 was considered to be significant.

RESULTS

Effects of NPY intracerebroventricular injection on sleep. Intracerebroventricular injection of NPY elicited decreases in NREMS and REMS in the first hour after injection (Fig. 2). The lowest dose (0.4 µg NPY) had a statistically significant effect on NREMS, as indicated by ANOVA (Table 1), that was
confined to the third hour after the injection (SNK test, Fig. 2); the biological significance of this isolated difference in NREMS between the baseline and experimental day is questionable. There was no significant effect on the total episode number and episode duration of NREMS and REMS and on EEG SWA after the 0.4-μg dose of NPY (Table 2 and Fig. 2). The detailed analysis of EEG power spectrum in the first 3 h revealed significant decreases in the NREMS power spectrum in the 0.5- to 4.5-Hz frequency band; wake and REMS EEG were not affected (Fig. 3). There was no significant change in the food intake of the rats in response to 0.4 μg NPY (Fig. 4).

Administration of 2 μg NPY had significant effects on both NREMS and REMS, as indicated by ANOVA (Table 1). The effects were confined to the first hour; NREMS decreased from a baseline of 26.6 ± 2.2 to 12.6 ± 2.3 min after NPY treatment. REMS virtually disappeared in hour 1 on the test day. The reduced time spent in sleep may have resulted from a significant decrease in the average duration of NREMS episodes and a significant decrease in the number of REMS episodes (Table 2). There was a tendency toward decreased NREMS episode number, but statistical analyses did not show significant differences. The EEG SWA did not change significantly. Detailed analysis of the EEG showed a significant increase in EEG power spectrum in the 4- to 7-Hz frequency band during wake and REMS (Fig. 3). NPY (2 μg) significantly increased the food intake of the rats (Fig. 4).

The 10-μg NPY injection was also followed by a significant decrease in both NREMS and REMS amount, as indicated by ANOVA (Table 1). Post hoc analyses showed significant suppression in NREMS in hour 1. The NREMS decrease may be because of the significant decrease in the number of NREMS episodes; the changes in average NREMS episode duration were not significant. In hour 1, on the baseline day, rats already had a minimal amount of REMS, and on the NPY day, they had no REMS at all. Injection of 10 μg NPY did not change the EEG SWA. The EEG power spectra did not show
Table 1. Effects of icv and lateral hypothalamic administration of NPY on NREMS, REMS amount, EEG SWA, and EEG power spectra of vigilance states.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>F (df)</th>
<th>P</th>
<th>Treatment Interaction</th>
<th>F (df)</th>
<th>P</th>
<th>Treatment Interaction</th>
<th>F (df)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPY, 1µg in icv</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NREMS amount</td>
<td>1.374</td>
<td>0.246</td>
<td>1.121</td>
<td>0.302</td>
<td>0.586</td>
<td>1.551</td>
<td>0.218</td>
<td>0.643</td>
</tr>
<tr>
<td>REMS amount</td>
<td>0.021</td>
<td>0.901</td>
<td>0.496</td>
<td>0.477</td>
<td>0.494</td>
<td>0.576</td>
<td>0.444</td>
<td>0.507</td>
</tr>
<tr>
<td>Wake power spectrum</td>
<td>0.782</td>
<td>0.378</td>
<td>0.782</td>
<td>0.378</td>
<td>0.378</td>
<td>0.782</td>
<td>0.378</td>
<td>0.378</td>
</tr>
<tr>
<td>NREMS power spectrum</td>
<td>0.325</td>
<td>0.572</td>
<td>0.325</td>
<td>0.572</td>
<td>0.572</td>
<td>0.325</td>
<td>0.572</td>
<td>0.572</td>
</tr>
<tr>
<td>REMS power spectrum</td>
<td>0.036</td>
<td>0.853</td>
<td>0.036</td>
<td>0.853</td>
<td>0.853</td>
<td>0.036</td>
<td>0.853</td>
<td>0.853</td>
</tr>
</tbody>
</table>

Effects of NPY lateral hypothalamic injection on sleep. The effects of NPY lateral hypothalamic injections on sleep and feeding were similar to those observed after intracerebroventricular treatment (Fig. 1). Time spent in NREMS and REMS was decreased significantly in hours 1 and 3, respectively (Table 1). NREMS episode number significantly decreased in hour 1, and there was a tendency toward a decrease in average NREMS episode duration as well (Table 2). EEG SWA increased in response to the injection starting from hour 3; however, post hoc analyses did not show significance in any particular hour. The detailed analyses of EEG power spectra showed a slight but statistically significant increase of the EEG power spectrum in the 6- to 7.5-Hz frequency band during the wake period (Fig. 2). Food intake was enhanced significantly by lateral hypothalamic injection of NPY (Fig. 4).

**DISCUSSION**

Intracerebroventricular and lateral hypothalamic administration of NPY suppressed NREMS and REMS in rats when injected at light onset. In addition, it also stimulated food intake in the first hour after both routes of administration. NPY is primarily implicated in feeding regulation. Our findings confirm previous studies that central injection of NPY increases food intake in rats (24, 39).

Previous reports concerning NPY’s sleep-modulating effect did not yield consistent results. In rats, intracerebroventricular injection of NPY 3 h after light onset failed to change the amount of time spent in slow-wave sleep (11); the differences in the results between that study and current one may be attributed to the different time of injection. In humans, repeated intravenous bolus injections of NPY during the dark period promoted NREMS and had no effect on sleep EEG spectra in normal young male subjects (3). The same research group carried out a more recent study in older male and female patients with depression (15). NPY infusion caused the shortening of NREMS and REMS latencies but did not affect the time spent in stage 2 sleep, slow-wave sleep, REMS, or total sleep time. There was no significant difference in the responsiveness to NPY between the depressed and control groups. In our experiments, when NPY was injected at light onset, the sleep-suppressive effects were robust; both NREMS and REMS decreased significantly in the first hour of the light period, and REMS practically disappeared. The decrease in NREMS amount in the first hour after the injection is clearly reflected in the decreased total number of NREMS episodes; nevertheless, there was a tendency toward decreased average duration of NREMS episodes as well. After NPY injection, REMS completely disappeared in the first hour of the light period; however, the amount of REMS on the baseline day was also relatively low.

The mechanism through which NPY promotes wakefulness is unknown. NPY-immunoreactive cell bodies are present in the ARC, PVN, SCN, DMH, and LH, nuclei implicated in feeding and sleep-wake regulation. NPY Y1 and Y5 receptors, which are mainly involved in the food intake stimulatory activity of NPY, are also present in these hypothalamic nuclei.

icv, Intracerebroventricular; NPY, neuropeptide Y; LH, lateral hypothalamus; NREMS, non-rapid-eye-movement sleep; REMS, rapid-eye-movement sleep; SWA, slow-wave activity; EEG, electroencephalogram. *P < 0.05, significant difference between baseline and treatment condition. NS, nonsignificant difference between baseline and treatment condition.
NPY-containing axon terminals innervate orexinergic neurons in the LH. Intracerebroventricular injection of NPY increases c-fos immunoreactivity in the ARC, PVN (25), and lateral hypothalamic orexinergic neurons (6). Besides stimulating feeding, orexins promote wakefulness and locomotor activity (36); therefore, it is also possible that NPY’s stimulatory action on orexinergic cells in the LH mediates the wake-promoting effect of NPY. This notion is supported by our observation that NREMS decreased in response to lateral hypothalamic injection of NPY. A reciprocal relationship exists between NPY and orexinergic neurons. Intracerebroventricular administration of orexins stimulates NPY expression in

Table 2. Total NREMS and REMS episode number and the average NREMS and REMS episode duration after icv and lateral hypothalamic administration of NPY in the first hour after the injections

<table>
<thead>
<tr>
<th></th>
<th>NREMS Episode No.</th>
<th>Average NREMS Episode Duration, min</th>
<th>REMS Episode No.</th>
<th>Average REMS Episode Duration, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>6.8±0.8</td>
<td>4.4±0.5</td>
<td>0.6±0.3</td>
<td>1.6±0.2</td>
</tr>
<tr>
<td>NPY (0.4 μg)</td>
<td>8.4±1.1</td>
<td>4.4±0.6</td>
<td>1.3±0.4</td>
<td>1.9±0.4</td>
</tr>
<tr>
<td>Baseline</td>
<td>7.2±0.7</td>
<td>4.0±0.5</td>
<td>1.5±0.4</td>
<td>2.3±0.3</td>
</tr>
<tr>
<td>NPY (2 μg)</td>
<td>5.2±1.1</td>
<td>2.5±0.4*</td>
<td>0.1±0.1*</td>
<td>NA</td>
</tr>
<tr>
<td>Baseline</td>
<td>8.1±0.6</td>
<td>3.1±0.3</td>
<td>0.4±0.3</td>
<td>NA</td>
</tr>
<tr>
<td>NPY (10 μg)</td>
<td>4.4±0.9*</td>
<td>2.6±0.8</td>
<td>0.0±0.0</td>
<td>NA</td>
</tr>
<tr>
<td>Baseline</td>
<td>6.9±0.9</td>
<td>4.5±0.8</td>
<td>0.5±0.3</td>
<td>NA</td>
</tr>
<tr>
<td>LH (2 μg NPY)</td>
<td>3.8±1.3*</td>
<td>2.4±0.7</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05, significant difference between baseline and treatment condition. NA, not available.

Fig. 3. EEG power spectra of wake, NREMS, and REMS in the first 3-h time block after icv and lateral hypothalamic administration of NPY and PFS. See legend to Fig. 2 for details.
feeding. There is a growing body of evidence suggesting that exclusively because it enhances a competitive behavior such as is possible that NPY, similarly to ghrelin, suppresses sleep not NPY in food-deprived rats was not examined; nevertheless, it had no access to food. In the present study, the sleep effect of activity because ghrelin also induced wakefulness in rats that was not a direct consequence of its food intake-promoting previous study, the wakefulness-enhancing activity of ghrelin conditions (“dark-onset syndrome”). Orexins, NPY, and ghrelin are parts of the hypothalamic food intake regulatory circuit. It is possible that the activation of the same circuit results in wakefulness, which is independent, at least in part, from the food intake-inducing actions. Additional evidence suggesting the role of NPY in sleep regulation include that chronic REMS deprivation increases NPY expression in the rat hypothalamus (21) and NPY-like immunoreactivity shows a diurnal rhythm in the SCN and ARC, with a significant peak before onset of the dark period (18). We hypothesize that increasing hypothalamic NPY levels may be responsible, at least in part, for triggering behavioral changes characteristic of the dark-onset syndrome.

GRANTS
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