Orexin-induced feeding requires NMDA receptor activation in the perifornical region of the lateral hypothalamus

Dolores F. Doane,1 Marcus A. Lawson,2 Jonathan R. Meade,2 Catherine M. Kotz,3 and J. Lee Beverly1,2

1Division of Nutritional Sciences, 2Department of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, Illinois; and 3Veterans Affairs Medical Center Geriatric Research Education Clinical Center and Minnesota Obesity Center, Minneapolis, Minnesota

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Doane DF, Lawson MA, Meade JR, Kotz CM, Beverly JL. Orexin-induced feeding requires NMDA receptor activation in the perifornical region of the lateral hypothalamus. Am J Physiol Regul Integr Comp Physiol 293: R1022–R1026, 2007.—Food intake is stimulated following administration of orexin-A into the perifornical region of the lateral hypothalamus (LH/PFA). Orexin neurons originating in the LH/PFA interact with a number of hypothalamic systems known to influence food intake, including glutamatergic neurons. Glutamatergic systems in the LH/PFA were demonstrated to initiate feeding through N-methyl-D-aspartic acid (NMDA) receptors. Male Sprague-Dawley rats fitted with brain guide cannulas to the LH/PFA were used in two experiments. In the first experiment, a combination microdialysis/microinjection probe was used to deliver artificial cerebrospinal fluid (aCSF) or 500 pmol of orexin-A into the LH/PFA. Orexin-A increased interstitial glutamate to 134 ± 12% of baseline (P < 0.05), which remained elevated over the 120-min collection period. In the second experiment, the NMDA receptor antagonist di-2-amino-5-phosphonopentanoic acid (d-AP5; 10 nmol) was administered before orexin-A. The orexin-induced increase in food intake (from 1.1 ± 0.4 to 3.2 ± 0.5 g, P < 0.05) during the first hour was absent in rats receiving d-AP5 alone. These data support glutamatergic systems in the LH/PFA mediating the feeding response to orexin-A through NMDA receptors.

Food intake regulation; hypocretin; microdialysis

Since their discovery in the late 1990s, the neuropeptides orexin-A and orexin-B have been shown to increase food intake (29, 30). These neuropeptides arise from neurons in the subthalamic area, specifically the perifornical region of the lateral hypothalamus (LH/PFA) (7, 29), a brain area known to play a role in feeding behavior (2). Both peptides are effective in increasing food intake, but orexin-A was more effective in stimulating feeding when administered directly into the LH/PFA (8, 36, 39). In addition to their effects on food intake, the orexins influence arousal state and locomotor activity (12), which likely contributes to their effect on food intake (24, 35) and is in accord with orexin’s role in food anticipatory activity (22, 23). Physiologically relevant increases in either glucose or leptin reduced the activity of orexin neurons (6, 30, 45), suggesting that the metabolic state of the animal influences orexigenic pathways. How the orexins stimulate food intake is unclear, but it is likely to occur through interaction with neural systems known to influence feeding. Orexin neurons from the LH/PFA terminate on neuropeptide Y (NPY)-containing neurons (15), and exogenous orexin activated NPY neurons in the arcuate nucleus (24, 44). Conversely, administration of NPY increased fos-like immunoreactivity in orexin-positive neurons (25). Fos expression in orexin neurons was also increased in response to exogenous application of either ghrelin or galanin-like peptide, and immunoreactive nerve fibers for both peptides are in direct contact with orexin neurons in the LH/PFA (17, 42). A role for opioid pathways, although not in the LH/PFA directly, was also recently demonstrated (37).

Orexin was demonstrated to increase glutamatergic activity in several brain areas innervated by orexin neuronal fibers, including the LH/PFA (11, 20). Glutamate and orexin are colocalized within fibers in the tuberomamillary nucleus (41) and hypothalamus (28), and the postsynaptic action of orexin to stimulate pyramidal cells in the prefrontal cortex was facilitated by glutamate in a synergistic fashion (31). In addition, glutamate release was increased in response to systemic administration of orexin-A in both the locus coeruleus (18) and amygdala (16). Application of orexin to hypothalamic slices increased synaptic activity of glutamatergic interneurons in both the medial and lateral hypothalamus (43). Van den Pol et al. (43) proposed that orexin may act presynaptically through GABA or glutamate circuits in the arcuate nucleus to influence energy intake.

We chose to evaluate orexin’s effects on amino acid neurotransmitter release in the LH/PFA. Orexin administered into the LH/PFA stimulates a profound increase in food intake (8, 36, 39), and glutamate circuits in the LH/PFA have been conclusively demonstrated by Stanley et al. (32–34) to stimulate feeding. Glutamate was recently demonstrated to mediate NPY-induced feeding in this same brain region (19). In a recent report (38), we demonstrated a modest decline in GABA release in the LH/PFA in response to local orexin application. The objective of the present study was to monitor glutamate and orexin release in the LH/PFA following administration of a dose of orexin-A that stimulates food intake.

MATERIALS AND METHODS

All procedures used in this study were approved by the Institutional Animal Care and Use Committee of the University of Illinois. Male Sprague-Dawley rats (Charles River Laboratories), 250–300 g, were housed singly in Plexiglas cages (30 × 30 × 38 cm) in a light (12:12-h light-dark cycle; lights on at 0700) and temperature (26 ± 2°C)-controlled room. Fresh water and rodent diet (Harlan Teklabs, Madison, WI) were available at all times, except where stated otherwise.

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Experiment 1. Surgical and sampling procedures were as previously described (38). Briefly, rats \((n = 20)\) were anesthetized with a mixture of ketamine HCl, xylazine HCl, and acepromazine (30:6:1 mg/kg im) before a unilateral guide cannula was stereotaxically positioned and fixed in position 2 mm above the LH/PFA (2.1 mm posterior, 1.8 mm lateral, and 6.3 mm dorsal from bregma) using the stereotactic atlas of Paxinos and Watson (26). Rats were monitored until completely recovered from the anesthesia and postsurgical analgesia provided by Banamine (1.5 mg/kg sc). Following recovery, a combination microinjection/microdialysis probe with 0.20 × 1-mm cuprophan membrane (AKZO-Nobel, Wuppertal, Germany) was placed into the LH/PFA at least 3 h before samples were collected. To minimize stress, samples were collected from unrestrained animals in their home cages. To minimize confounding influences of activity and feeding, food was removed during the microdialysis period and samples were collected during the mid-light phase. The microdialysis probe was connected by fused silica lines through a liquid swivel (Instech, Plymouth Meeting, PA) to a 1-ml gas-tight syringe on a microinfusion pump (CMA Microdialysis, North Chelmsford, MA) and continuously perfused with Krebs-Ringer buffer (in mM: 147 NaCl, 4 KCl, and 2.4 CaCl\(_2\), pH 6.4) at 2.0 µl/min. Dialysate was collected into chilled microtubes at 10-min intervals. A single administration (0.5-µl injection, 0.1 µl/min) of artificial cerebrospinal fluid (aCSF) or 500 pmol of orexin-A (Phoenix Pharmaceuticals, Belmont, CA) was administered through the injection portion of the probe 30 min after start to collect samples. Samples were stored at −81°C until assayed for glutamate.

Food was returned after the last sample had been collected, and intake was measured after 60 min (from time 120 to 180 min postinjection). In a preliminary trial, rats receiving this dose of orexin-A ate nearly twice as much as control rats \((2.7 ± 0.3 \text{ vs. } 1.4 ± 0.4 \text{ g/2 h}; P = 0.02)\). Cannula placements were verified histologically at the end of the study.

Glutamate was analyzed by HPLC using an ESA Coulochem II analyzer (E1 = +200 mV and E2 = +650 mV) with a 5020 guard cell \((G1 = +700 \text{ mV})\) following derivatization with OPA/BME (0.27% o-phthalic dicanboxaldehyde, 10% methanol, 0.5% β-mercaptoethanol, and 0.1 M sodium tetraborate, pH 9.3) and separation on a reverse phase 50 × 3-mm C18 (3 µm) Shiseido Capcell Pak MG column (ESA, Chelmsford, MA). The mobile phase \((\text{pH 6.7})\) consisted of 100 mM Na2HPO\(_4\) in 20% methanol. The chromatograms were integrated and quantified using Dynamax MacIntegrator II software (Rainin Instruments, Woburn, MA). The interassay coefficient of variation was ≤3%.

Experiment 2. The N-methyl-d-aspartic acid (NMDA) receptor antagonist d-2-amino-5-phosphonopentanoic acid (d-AP5) was used to determine whether the orexin-A-induced increase in glutamate mediated the increased food intake by using an experimental design described by Lee and Stanley (19). Rats \((n = 12)\) fitted with bilateral injection guide canulae stereotaxically positioned to be 1 mm dorsal to the LH/PFA were randomly assigned to a treatment group in a 2 × 2 factorial design (main effects: orexin-A and d-AP5) following recovery. Each rat received a unilateral injection of either 0.3 µl of aCSF or d-AP5 (10 nmol,0.3 µl of aCSF, 0.1 µl/min) followed 10 min later by a unilateral injection of aCSF or 500 pmol of orexin-A (0.5-µl injection, 0.1 µl/min) into the same side. Food intake was measured 1, 2, and 3 h after injections. One week later, animals received the opposite combination into the contralateral LH/PFA (e.g., rats that had received aCSF/aCSF received d-AP5/orexin-A, and vice versa, \(n = 6\) per group). Cannula placements were verified histologically at the end of the study (Fig. 1).

Data analysis. Data from the first experiment were analyzed using repeated-measures ANOVA and Scheffé’s post hoc test with \(α\) levels set at 0.05. Changes from baseline within a treatment group were determined by paired \(t\)-test. In the second experiment, data were analyzed using two-way ANOVA (main effects: orexin-A and d-AP5) and Bonferroni’s post hoc test. Ketamine, acepromazine, and Bana-

Fig. 1. Sampling and injection sites in experiments 1 and 2. Bars on left reflect positions of 1-mm microdialysis membranes in experiment 1. Diamonds on right reflect positions of injection cannula tips in experiment 2. Schematics are from Ref. 26.
is consistent with glutamate mediating the feeding effect of orexin-A. The observed increase in glutamate is similar in magnitude and duration reported in the locus coeruleus (18) and the amygdala (16). We do not know the source of the glutamate measured; however, it is likely from local glutamatergic interneurons or possibly from orexin neurons. The activity of glutamatergic neurons was increased presynaptically by orexin in lateral hypothalamic brain slices (11). Collaterals from orexin axons apparently make synaptic contact with other orexin neurons in the hypothalamus (15), and many of these neurons display glutamatergic immunoreactivity (20, 28, 41).

In addition, vesicular glutamate transporters are expressed by orexin neurons in the LH (28, 31). Both Li et al. (20) and Song et al. (31) have suggested that glutamate interacts synergistically with orexin. The increased interstitial glutamate may reflect feedback from orexin-stimulated circuits; however, in increased glutamatergic activity was also measured in cell culture and thin (e.g., 200 μM) brain slice preparations in which such circuits are unlikely to be intact (11, 15, 20, 43). Thus the increased interstitial glutamate in the present study is most likely a direct response to the administered orexin-A.

The increased food intake in the present study is similar to reports by investigators using a similar dose of orexin-A (36, 39). Food intake was increased in a dose-dependent manner following administration of orexin-A (8, 36, 39), which is more effective in stimulating food intake than orexin-B (8). Orexin receptor 1 (OX-R1) and 2 (OX-R2) are both present in the LH/PFA and bind orexin-A (1, 21, 29). We are unaware of any studies using an OX-R2 antagonist; however, elevated feeding after exogenous orexin-A (administered intracerebroventricularly) was blocked by an OX-R1 antagonist (14). Consistent with earlier reports (38, 40), the effect of orexin-A on food intake was most vigorous during the first hour after administration, with no effect after 2 h. However, termination of the orexigenic effect may require food consumption. In the first experiment, food was not available until 2 h after orexin-A administration and food intake was higher than controls over the next hour. The continued desire to eat may have been mediated, in part, by the continued increase in glutamate release more than 60 min after orexin-A was administered. The increase in GABA release occurring in the ventromedial hypothalamus after a glucoprivic challenge remained elevated until rats were allowed to eat (3). We did not measure glutamate release when food was available, in part because of the confounding effects of feeding on glutamate release in the LH (27). No food was consumed during the present microdialysis study (experiment 1), so the increase in glutamate we measured was not a consequence of eating. However, glutamate activation with food presentation would be consistent with glutamate mediating orexin’s putative role in maintaining arousal and reward (reviewed in Ref. 13).

The result of the second experiment, to block NMDA receptors at the time of orexin-A administration, confirms that the feeding response to orexin-A requires glutamatergic activation in the LH/PFA. In this brain area, glutamate has consistently been demonstrated to promote a robust feeding response (32–34) that is mediated by NMDA receptors in the area (9, 10, 33). In an experiment of similar design to experiment 2, Lee and Stanley (19) were able to block the increase in feeding after NPY administration into the LH/PFA by prior injection of N-AP5 into the same brain site. In the present study, providing 10 nmol of N-AP5 completely blocked the increased food intake to local orexin-A administration. This dose of N-AP5 was based on work by reports that 1 nmol was ineffective in blocking the increase in food intake by exogenous NPY (19) or NMDA (33) administered into the LH/PFA. A 5-nmol dose of N-AP5 administered into the LH/PFA was ineffective in preventing the feeding response to orexin-A (Kotz CM, unpublished data). There was no effect of our unilateral injection of N-AP5 alone to reduce food intake. However, rats did have reduced intakes following bilateral injection of the same N-AP5 dose into the lateral hypothalamus (4, 33).

In addition to the increase in glutamate release, other neurotransmitter systems are likely influenced by injection into the LH/PFA. Although orexin was reported to activate GABAergic interneurons in the arcuate nucleus (43), we recently reported a modest decrease in GABA release in the LH/PFA under the same conditions as reported presently. These results suggest there is a complimentary increase in the excitatory amino acid glutamate and decrease in the inhibitory amino acid GABA in the LH/PFA following local increases in orexin-A.
Interestingly, interfering with either the reduction in GABA tone, by administering the GABA receptor agonist muscimol (38), or increased glutamatergic activity, as in experiment 2, effectively blocked the feeding response to orexin-A. This would indicate that reciprocal changes in both circuits in the LH/PFA are required for the feeding response. We also cannot rule out the involvement of other hypothalamic feeding-related neuronal circuits. It is possible that the same feeding circuits activated by NPY in the Lee study were activated by orexin-A in the present study. Several reports describe the interconnection of orexin and NPY circuits in the hypothalamus (15, 24, 44). Exogenous NPY activated ~23% of orexin-positive neurons in the LH (25), and d-AP5 administered into the LH/PFA blocked NPY induced feeding (19). Thus NPY released in the LH/PFA may induce feeding by activating orexinergic neurons, which then affect the activity of local glutamatergic and GABAergic systems. This putative pathway may be a part of systems inducing feeding or may facilitate a specific aspect of feeding, such as foraging (5).

In summary, we report an increase in intristitial glutamate in the LH/PFA following local application of a dose of orexin-A that stimulates food intake. This increase in glutamate is consistent with reports that orexin-A increases glutamatergic activity in other brain regions. In the LH/PFA, glutamate was conclusively demonstrated to induce feeding NMDA receptors (32, 33). Application of the NMDA antagonist d-AP5 before orexin-A administration abolished the orexigenic response to orexin-A. These data provide further support for a role of endogenous orexins in food intake regulation, where their likely action is through interaction with other neuropeptidergic systems in the hypothalamus and mediated by local glutamatergic neurons.

GRANTS

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REFERENCES


