Neuroendocrine regulatory peptide-2 regulates feeding behavior via the orexin system in the hypothalamus

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Toshinai K, Yamaguchi H, Kageyama H, Matsuou T, Koshinaka K, Sasaki K, Shioda S, Minamino N, Nakazato M. Neuroendocrine regulatory peptide-2 regulates feeding behavior via the orexin system in the hypothalamus. Am J Physiol Endocrinol Metab 299: E394–E401, 2010. First published June 15, 2010; doi:10.1152/ajpendo.00768.2009.—Neuroendocrine regulatory peptides (NERPs) in the central regulation of feeding and energy homeostasis. We attempted to identify NERPs expressing neurons in rats by immunohistochemistry. We studied the effects of intracerebroventricular (icv) administration of NERP-2 on feeding, body temperature, oxygen consumption, and locomotor activity in rats and mice. Intracerebroventricular administration of NERP-2, but not NERP-1 or a form of NERP-2 bearing a COOH-terminal glycine extension, increased food intake in rats. We investigated the downstream signal of NERP-2 on the basis of studies of NERP-2-induced feeding with neutralization of orexins, neuropeptide Y, or agouti-related protein. NERP-2 expression localized to the lateral hypothalamus (LH) and the dorsomedial perifornical hypothalamus in rats, colocalizing with orexins that activate feeding behavior and arousal. NERP-2 administration induced Fos protein, a marker of neuronal activation, in the orexin-immunoreactive neurons. Vgf mRNA levels were upregulated in the rat LH upon food deprivation. Intracerebroventricular administration of NERP-2 also increased body temperature, oxygen consumption, and locomotor activity in rats. Treatment with anti-NEPR-2 IgG decreased food intake. NERP-2-induced bioactivities could be abrogated by administration of anti-orexins IgG or orexin receptor antagonists. NERP-2 did not induce food intake or locomotor activity in orexin-deficient mice. Our findings indicate that hypothalamic NERP-2 plays a role in the control of food intake and energy homeostasis via the orexin pathway. Thus, VGF serves as a precursor of multiple bioactive peptides exerting a diverse set of neuroendocrine functions.

hypothalamic peptide; VGF protein; feeding regulation; energy homeostasis; neuronal activation

FEEDING IS PRECISELY REGULATED by multiple redundant pathways utilizing various substances produced both in the brain and the peripheral organs. The hypothalamus plays a central role in the integrated regulation of feeding and energy homeostasis (reviewed in Refs. 6 and 26). Two hypothalamic regions are involved primarily in feeding regulation. The arcuate nucleus (ARC) contains populations of neurons expressing the orexigenic factors neuropeptide Y (NPY) and agouti-related protein (AgRP) (23, 36) and the anorexigenic factors proopiomelanocortin and cocaine-amphetamine-regulated transcript (21). The lateral hypothalamus (LH) expresses the orexigenic peptides orexin-A and -B (33) [also designated hypocretin-1 and -2 (9)] and melanin-concentrating hormone (MCH) (30). These hypothalamic orexigenic and anorexigenic factors communicate with the gut, adipose tissue, hormones, and other regions of the central nervous system to regulate appropriate energy balance.

Endogenous ligand screening using cell lines expressing orphan G protein-coupled receptors (GPCRs) in combination with genetic engineering techniques has greatly expanded our understanding of the feeding regulatory network. Several hypothalamic peptides that regulate energy homeostasis, including orexin (33), ghrelin (20), and neuropeptide W (40), have been identified as ligands for orphan GPCRs using this method. To facilitate the identification of bioactive peptides, we profiled all secretory peptides produced by human medullary thyroid carcinoma TT cells and identified potential candidate bioactive peptides as those with COOH-terminal amidation, a postranslational modification common to multiple bioactive peptides (46). We identified two novel amidated peptides, which we designated human neuroendocrine regulatory peptide (NERP)-1 and -2 according to their localization and physiological role. Human NERP-1 is 26 amino acids in length, whereas rat NERP-1 is 25 amino acids in length. Human and rat NERP-2 are both 38-amino acid peptides. Immunohistochemical staining using an antibody specific for NERP-1 or NERP-2 revealed that NERPs were expressed in the paraventricular nucleus and supraoptic nucleus of the hypothalamus, colocalizing with vasopressin in storage granules. These peptides were also found to modulate vasopressin (antidiuretic hormone) release (46). NERPs are derived from distinct regions of the neurosecretory protein VGF, which was identified originally as a product of the nerve growth factor-responsive gene vgf in rat pheochromocytoma PC12 cells (22). Homozygous vgf-deficient mice are lean with hypermetabolic rates compared with wild-type and heterozygous littermates (13), suggesting that full-length vgf also encodes an orexigenic or anabolic peptide. However, a VGF-derived peptide having such bioactivity has yet to be identified.

In this study, we demonstrated that NERP-2 colocalizes in rats with orexins, a family of neuropeptides that stimulate feeding behavior and enhance energy expenditure via stimulation of sympathetic nerve activity, in the lateral hypothalamus. We have shown that NERP-2, but not NERP-1, stimulates
feeding in rats and mice and regulates energy homeostasis by acting via the orexin system.

**MATERIALS AND METHODS**

**Animals.** Male Wistar rats (aged 9–10 wk; Charles River Laboratories, Yokohama, Japan) as well as orexin-deficient mice [kindly donated by T. Sakurai (4)] and their wild-type littermates (aged 12–14 wk) were maintained in individual cages under controlled temperature (21–23°C) and light (lights on 0800–2000) conditions. Animals were given ad libitum access to food and water. An intracerebroventricular (icv) cannula was implanted into the lateral cerebral ventricle as described (27). Proper placement of the cannulae was verified upon completion of the experiment by dye administration. Only animals demonstrating progressive weight gain after surgery were used in subsequent experiments. Experimental peptides were dissolved in artificial cerebrospinal fluid (aCSF) (46). All animals were acclimated to handling procedures of the administration before peptide administration. Peptides were administered 1 wk after surgery. Experiments were conducted using crossover methods in which all animals received either test peptide or vehicle alone as a control on separate days. All animal experiments were performed in accordance with the Japanese Physiological Society’s guidelines for animal care and were approved by the Animal Care and Use Committee of the University of Miyazaki.

**Peptide synthesis and antibody preparation.** NERP-1, NERP-2, and a form of NERP-2 bearing a COOH-terminal glycine (Gly) extension (NERP-2-Gly) were synthesized as described (46). Anti-NERP-2 antibodies were produced in rabbits as described (46). Briefly, a cysteiny1 COOH-terminal peptide derived from rat NERP-2 (CQGGARQRDLG-NH2) coupled to keyhole limpet hemocyanin was used to immunize rabbits. We purified anti-NERP-2 IgG from anti-NERP-2 antiserum as described (8).

**Quantitative RT-PCR.** The LH was punched out from brain slices obtained from three rat groups; those fed ad libitum, those fasted for 1 wk, and those fasted for 1 wk and then refed for 2 h (n = 4/group). Total RNA was extracted from the LH with TRIzol Reagent (Invitrogen, Carlsbad, CA). Quantitative RT-PCR for VGF was performed using a LightCycler system (Roche Diagnostics, Mannheim, Germany) with a LightCycler Fast Start DNA Master SYBR Green I kit (Roche Diagnostics), using the following primer set for rat vgf mRNA: 5′-CATCGGCTCTCTCTCCTGTT-3′ and 5′-GAAGAGCTCTCC-CTGTTCTC-3′ (GenBank accession no. M74223).

**Feeding experiments.** Unless otherwise specified, we measured 1-h food intake. Rats fed ad libitum (n = 10–15/group) received an icv injection of rat NERP-1, NERP-2, NERP-2-Gly (0.1–5 nmol), or aCSF alone at 1000. To investigate the role of endogenous NERP-2, anti-NERP-2 IgG (0.5 μg) or normal rabbit serum (NRS) IgG (0.5 μg) was administered icv at 1700 to rats fed ad libitum (n = 8/group), followed by measurements of food intake at 1, 2, 4, and 24 h after the beginning of the dark phase. To assess the effect of other administration methods, 100 μl of saline with NERP-2 (5 nmol) or ghrelin (5 nmol; Peptide Institute, Osaka, Japan) was administered intraperitoneally (ip) to rats (n = 8/group) at 1000. To assess the involvement of orexin in NERP-2-induced feeding, anti-orexin-A and anti-orexin-B IgGs (0.25 μg each) (8) or anti-MCH IgG (0.5 μg; Phoenix Pharmaceuticals, Burlingame, CA) were injected icv into rats (n = 12/group) 3 h before icv NERP-2 administration (1 nmol) at 1100. To block orexin-induced feeding mediated by the orexin 1 receptor (OX1R) (14), rats (n = 6/group) were injected icv with an OX1R antagonist, SB-408124 (1 μg/0.5 μl 20% DMSO solution; Sigma-Aldrich, St. Louis, MO), 3 h prior to icv administration of NERP-2 (1 nmol) or MCH (1 nmol; Peptide Institute) at 1100. To examine the role of orexin in NERP-2 bioactivity, we gave orexin-deficient mice and their wild-type littermates (n = 8/group) an icv injection of 2 μl of aCSF containing either NERP-2 (1 nmol) or MCH (1 nmol) at 1000. To assess the interaction between orexin and NERP-2, we injected 10 μl of aCSF with orexin-A (1 nmol; Peptide Institute), NERP-2 (1 nmol), orexin-A plus NERP-2 (each 1 nmol), MCH (1 nmol), or MCH plus NERP-2 (each 1 nmol) icv to rats fed ad libitum (n = 7/group) at 1000. Rats (n = 6/group) were also treated by icv injection with 5 μl of 20% DMSO solution containing both an NPY Y1 receptor antagonist, GR231181 (12.5 μg; Sigma-Aldrich), and a Y5 receptor antagonist, CP71683A (60 μg; Sigma-Aldrich) (3, 50), 1 h before icv administration at 1000 of 1 nmol of NERP-2, NPY (Peptide Institute), or AgRP96-132 (Peptide Institute); we then measured 2-h food intake. As a control, rats (n = 4–6/group) were injected icv with anti-AgRP IgG (0.5 μg; Phoenix Pharmaceuticals) at 0900, 3 h before icv administration of 1 nmol of NERP-2 or AgRP96-132. To investigate its suppressive effect on feeding, anti-AgRP IgG (0.5 μg) was injected icv into six rats at 1900. We measured 2-h food intake in all studies using AgRP (19). Peptides and antibodies for icv administration to rats were dissolved in 5 μl of aCSF unless otherwise specified.

**Immunohistochemistry.** To perform immunofluorescent staining of the LH, brains were removed from rats treated with 200 μg of cholerae following perfusion with 2% paraformaldehyde (43). For single immunofluorescence staining, sections were incubated for 2 days at 4°C with an antibody against rat NERP-2 (1:1,500). Antibody binding was detected by incubating brain sections in Alexa 546-labeled goat anti-rabbit IgG for 1.5 h. All Alexa dye-conjugated IgGs, used at 1:400, were purchased from Molecular Probes (Eugene, OR). For double immunofluorescence staining of the LH, brain sections initially treated as described above with a combination of anti-rat NERP-2 antibody and Alexa 564-labeled goat anti-rabbit IgG were then incubated at 4°C for an additional 2 days with goat anti-orexin-A (1:1,000; Santa Cruz Biotechnology, Santa Cruz, CA) or chicken anti-MCH (1:2,000; Chemicon International, Temecula, CA) antibodies. Sections were then incubated with Alexa 488-labeled donkey anti-goat or goat anti-chicken IgG. All samples were visualized using an Olympus AX-70 fluorescence microscope (Olympus, Tokyo, Japan). To determine the frequency with which NERP-2 and orexin-A colocalized, we captured dual immunofluorescence images using an A1si wide-band spectral confocal laser microscope with a spectral-unmixing system equipped with 488-nm diode-pumped solid-state lasers and 561-nm diode-pumped solid-state lasers integrated into an Eclipse Ti microscope using a Plan Apochromat 10× NA 0.45 and 20× VCNA 0.75 objective (Nikon, Tokyo, Japan). Image acquisition was performed using NIS-Elements software (Nikon). Control studies used an anti-NERP-2 antibody preabsorbed with 10 μg of rat NERP-2.

**Fos expression.** NERP-2 (1 nmol/5 μl aCSF), orexin-A (1 nmol/5 μl aCSF), or NERP-2-Gly (1 nmol/5 μl aCSF) was injected icv into rats 90 min before transcardial perfusion with 4% paraformaldehyde fixative. For double immunofluorescence staining, hypothalamic sections were incubated for 2 days at 4°C with the combination of goat anti-orexin-A antibody and Alexa 488-labeled donkey anti-goat IgG. Sections were then incubated at 4°C for 2 days with the combination of rabbit anti-Fos antibody (1:1,500; Santa Cruz Biotechnology) and Alexa 546-labeled goat anti-rabbit IgG. We quantitated the number of neurons coexpressing Fos with orexin or MCH in two randomly selected visual fields in each of two sections from each rat by fluorescence microscopy.

**Orexin-A release from hypothalamic explants.** Six hypothalami were stimulated for 20 min each with rat NERP-2 (10−8 M) or NERP-2-Gly (10−8 M). Stimulation periods were separated by a 20-min recovery period. At the end of each experiment, these hypothalami were exposed to KCl (10−6 M) for 20 min to verify the viability of the hypothalami (42). Media aliquots were assayed by orexin-A RIA (7). All experiments were repeated six times.

**Locomotor activity.** To block orexin-induced locomotor activity mediated by both OX1R and the orexin 2 receptor (OX2R) (35), rats were given an icv administration of both an OX1R antagonist (SB-408124, 1 μg/2.5 μl 20% DMSO solution) and an OX2R antagonist (N-acetyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline; a kind gift of AJP-Endocrinol Metab • VOL 299 • SEPTEMBER 2010 • www.ajpendo.org

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Banyu Pharmaceuticals, Tokyo, Japan, 1 μg/2.5 μl 20% DMSO solution) and then injected icv with 1 nmol of NERP-2 or orexin-A. Orexin-deficient mice and wild-type littermates (n = 5/group) were injected icv with NERP-2 (1 nmol/2 μl aCSF) or vehicle alone. We then measured locomotor activity in sound- and light-proof cages equipped with infrared light beam detectors (Muromachi, Tokyo, Japan) as described (25). Locomotor activity counts, measured every 15 min, were summed from 30 to 90 min after compound administration.

Body temperature and oxygen consumption. We measured the body temperatures of rats at 15-min intervals from 15 to 120 min after icv administration of 5 nmol of NERP-1, NERP-2, or NERP-2-Gly (n = 8/group), as described elsewhere (25). Oxygen consumption was measured using an O2/CO2 Analyzer MM202R apparatus (Muromachi) (25). Rats (n = 5/group) were initially given an icv dose of NERP-2 (5 nmol), NERP-2-Gly (5 nmol), NERP-1 (5 nmol), orexin-A (1 nmol), or aCSF alone and then individually returned to sealed chambers. Oxygen consumption was measured over 180 min, during which rats were deprived of food and water. Next, we measured oxygen consumption during the dark phase of animals given anti-NERP-2 IgG (0.5 μg) or NRS IgG (0.5 μg) icv (n = 8/group) at 1700. Third, we measured oxygen consumption of rats (n = 5/group) 60 min before and 60 min after icv administration of orexin-A (1 nmol) or NERP-2 (5 nmol) under anesthesia with pentobarbital sodium.

Statistical analysis. All data are expressed as means ± SE. Data were analyzed initially by one-way or two-way ANOVA with a post hoc Fisher’s test. Comparisons between two groups were performed using the unpaired t-test. P values < 0.05 were considered to be statistically significant.

RESULTS

NERP-2 stimulated food intake. Vgf mRNA levels in the rat LH increased significantly following a 48-h fast, returning to basal levels after refeeding (Fig. 1A). Intracerebroventricular injection of NERP-2 to freely feeding rats increased food intake during the light phase in a dose-dependent manner; the lowest effective dose was 0.1 nmol (Fig. 1B). Neither NERP-1 nor NERP-2-Gly affected food intake (Fig. 1B). Food intake decreased significantly for ≥24 h after icv administration of anti-NERP-2 IgG compared with NRS IgG treatment (Fig. 1C). Whereas ip administration of ghrelin, a positive control functioning as an orexigenic peptide in the periphery (7), increased food intake, NERP-2 given ip did not alter food intake (Fig. 1D).

Localization of NERP-2 in the LH. We used antiserum specific for the COOH-terminal amide structures of NERPs, which did not exhibit cross-reactivity with peptides bearing the COOH-terminal Gly extension or VGF itself (46). Staining of rat brain sections revealed that NERP-2-immunoreactive neurons were observed around the fornix, including the LH and dorsomedial perifornical hypothalamus (Fig. 2A). Orexin-immunoreactive neurons were also observed within these two regions (Fig. 2B). Double immunofluorescence staining demonstrated that NERP-2 colocalized completely with orexin-A (Fig. 2, C–E), but not with MCH (Fig. 2D), within the LH and dorsomedial perifornical hypothalamus. An absorption test showed that pretreatment with rat NERP-2 abolished positive immunostaining of the antibody against NERP-2 (data not shown).

Downstream signal of NERP-2 in the hypothalamus. Intracerebroventricular administration of NERP-2 stimulated Fos, a marker of neuronal activation (31), in 30.6 ± 1.6% of orexin-immunoreactive neurons in the rat LH, but NERP-2-Gly did not (Fig. 3, A–D). Intracerebroventricular administration of orexin-A induced Fos in 29.1 ± 2.8% of orexin-immunoreactive neurons (Fig. 3E). NERP-2 increased orexin-A release after administration to rat hypothalamic explants, whereas NERP-2-Gly did not (Fig. 4). KCl administration at the end of each experiment stimulated orexin-A release, indicating that these hypothalami kept the viability. Intracerebroventricular administration of both anti-orexin-A and anti-orexin-B IgGs to rats 3 h prior to NERP-2 injection completely abrogated NERP-2-induced feeding, whereas anti-MCH IgG had no effect (Fig. 5A). Intracerebroventricular administration of an OX1R-selective antagonist also abolished NERP-2-induced feeding (Fig. 5B). MCH served as a positive control to demonstrate an orexigenic effect independent of the orexin path-
way (Fig. 5B). We verified the functional relationship between NERP-2 and orexin in orexin-deficient mice. Intracerebroventricular administration of NERP-2 increased food intake in wild-type littermates but not orexin-deficient mice, whereas MCH increased food intake equivalently in both (Fig. 5C).

Intracerebroventricular administration of orexin-A, NERP-2, or orexin-A and NERP-2 together increased food intake in rats (Fig. 5D), with no significant differences between the increased amounts of food intake in these groups. Coadministration of NERP-2 and MCH, which utilizes an independent pathway,
exhibited a significant additive effect on feeding. We investigated the functional relationship between NERP-2 and the orexigenic peptides NPY and AgRP, which are produced in the ARC, a target nucleus of orexin-induced feeding stimulation. Coadministration of selective antagonists of the NPY Y1 and Y5 receptors abrogated the increases in feeding induced by NERP-2 and NPY but did not affect feeding stimulated by AgRP, a positive control utilizing an independent pathway (Fig. 5E). Intracerebroventricular administration of anti-AgRP IgG suppressed food intake during the dark phase (Fig. 5F). Although pretreatment with anti-AgRP IgG abrogated AgRP-induced feeding, it did not alter NERP-2-induced feeding, indicating that the AgRP pathway is not involved in NERP-2-mediated stimulation of feeding (Fig. 5G).

Body temperature, oxygen consumption, and locomotor activity. Intracerebroventricular administration of NERP-2 increased body temperature significantly for 60 min after injection, whereas NERP-2-Gly and NERP-1 had no effect (Fig. 6A). NERP-2 and orexin-A increased systemic oxygen consumption, whereas NERP-2-Gly and NERP-1 did not (Fig. 6B). NERP-2 increased oxygen consumption in anesthetized rats from a mean baseline level of 5.94 to 6.70 ml/min, whereas orexin-A increased it from a mean baseline level of 5.97 to 8.63 ml/min. In contrast to NRS IgG, anti-NERP-2 IgG administration specifically suppressed oxygen consumption (Fig. 6C). Both NERP-2 and orexin-A increased locomotor activity in rats; two selective antagonists for OX1R and OX2R abrogated these increases (Fig. 6D). NERP-2 increased locomotor activity in wild-type littermates but not orexin-deficient mice (Fig. 6E).

DISCUSSION

We have demonstrated that NERP-2, a peptide processed from VGF, is a novel member of the subset of feeding regulatory peptides functioning in the hypothalamus. Bioactive peptides, cleaved from precursor proteins via limited cleavage by prohormone convertases, must often undergo posttransla-

Fig. 4. NERP-2-induced orexin release from the rat hypothalamus. Administration of NERP-2 (10^{-3} M), but not NERP-2-Gly (10^{-3} M), increased orexin-A release from the rat hypothalamus. *P < 0.05 vs. control vehicle and NERP-2-Gly.

Fig. 5. Functional downstream signal of hypothalamic NERP-2 in rats and mice. A: anti-orexin IgG, but not anti-MCH IgG, abolished NERP-2-induced feeding in rats. *P < 0.05. B: pretreatment with an orexin 1 receptor (OX1R) antagonist abolished NERP-2-induced but not MCH-induced feeding in rats. *P < 0.01. C: food intake by orexin-deficient mice and wild-type littermates following icv injection of NERP-2 or MCH. *P < 0.05. D: amounts of food intake following icv injection of NERP-2, orexin-A, orexin-A + NERP-2, MCH, or MCH + NERP-2 in rats fed ad libitum, NS, not significant. *P < 0.05. E: the effects of Y1 and Y5 antagonist coadministration on the increases in food intake induced by NERP-2, NPY, and AgRP. *P < 0.01; **P < 0.001. F: anti-AgRP IgG suppressed food intake during the dark phase. G: AgRP neutralization did not affect NERP-2-induced feeding. *P < 0.01. aCSF, artificial cerebrospinal fluid; AgRP, agouti-related protein.
tional modifications to acquire biological activity (5, 53). VGF, the precursor of NERPs, contains multiple paired basic amino acid residues, which previously identified this protein as a likely peptide precursor (44). NERPs were discovered by comprehensive analysis of COOH-terminally amidated peptides secreted by a thyroid medullary carcinoma cell line (46). Vgf mRNA was detected in endocrine cell subsets in the anterior and posterior pituitary glands, adrenal medulla, pancreas, and gastrointestinal tract of rats (38). Vgf mRNA is also expressed in the brain; it is particularly abundant in the hypothalamus, especially in the LH and the arcuate, periventricular, paraventricular, supraoptic, suprachiasmatic, and preoptic nuclei (34, 37, 38, 52). In this study, we demonstrated that vgf gene expression in the LH was upregulated upon fasting. The expression levels of orexigenic peptides, such as NPY, AgRP, MCH, and orexins, produced in the hypothalamus are typically increased in situations of negative energy balance (29, 33, 45). Whereas OX1R and OX2R function in regulating locomotor activity (35). Orexin-induced food intake is mediated at least in part by the activation of OX1R in NPY neurons in the ARC (26, 48). We demonstrated that pretreatment with an OX1R antagonist abrogated NERP-2-induced feeding. NERP-2, which is coexpressed by orexin neurons, may activate orexin neurons by stimulating orexin release in an autocrine or paracrine fashion. Orexin’s multifaceted actions are mediated by two receptors, OX1R and OX2R (also known as hypocretin receptors 1 and 2) (8, 31). OX1R functions to regulate feeding behavior (14), whereas both OX1R and OX2R function in regulating locomotor activity (35). Orexin-induced food intake is mediated at least in part by the activation of OX1R expressed in NPY neurons in the ARC (26, 48). We demonstrated that pretreatment with an OX1R antagonist abrogated NERP-2-induced feeding. NERP-2 did not exert an additive effect on orexin-induced feeding, these results suggest that NERP-2 stimulated feeding via the orexin-NPY pathway.

The LH integrates autonomic and limbic information to modulate visceral motor and sensory pathways, including those underlying feeding and arousal behaviors (18). Both orexins and MCH are synthesized specifically in the LH, but these peptides are produced in independent neurons (10). Orexins regulate sleep and wakefulness, energy homeostasis, emotion, reward behaviors, and autonomic functions (reviewed in Ref. 32). Orexin released from orexin nerve terminals binds to the OX1R expressed in orexin neurons to regulate its own activity in an autocrine or paracrine fashion (2, 15). We demonstrated that icv administration of NERP-2 activated orexin-immunoreactive neurons to stimulate feeding. NERP-2, which is coexpressed by orexin neurons, may activate orexin neurons by stimulating orexin release in an autocrine or paracrine fashion.

Fig. 6. Modulation of orexin’s bioactivities by NERP-2. A: body temperature increased following icv administration of NERP-2, but not NERP-2-Gly or NERP-1. *P < 0.05; **P < 0.001 vs. NERP-2-Gly and NERP-1. B: oxygen consumption in conscious rats increased after icv administration of NERP-2 or orexin-A, but not NERP-2-Gly or NERP-1. *P < 0.05; **P < 0.001. C: oxygen consumption was reduced by anti-NERP-2 IgG treatment. *P < 0.01. D: effects of OX1R and orexin 2 receptor antagonist coadministration on NERP-2- and orexin-A-induced increases in locomotor activities in rats. *P < 0.05; **P < 0.01. E: locomotor activity in orexin-deficient mice and wild-type littermates after icv NERP-2 administration. *P < 0.01.
ity; consequently, orexin-A-treated rats demonstrate normal weight gain (17, 49). Orexin-overexpressing mice were also resistant to diet-induced obesity likely mediated by the promotion of energy expenditure (11). Thus, orexin is not merely a bioactive peptide that functions only in feeding regulation. Intracerebroventricular administration of NERP-2 also increased locomotor activity in wild-type mice but not in orexin-deficient mice or rats administered antagonists of both OX1R and OX2R; these results indicate that NERP-2 increases locomotor activity via the orexin pathway. Orexin-A increased oxygen consumption in anesthetized rats by 13.4% independently of locomotor activity (51). NERP-2 increased oxygen consumption by 0.91 ml/min in conscious rats and 0.76 ml/min in anesthetized rats, indicating that NERP-2 also increases oxygen consumption independently of locomotor activity. NERP-2 induced increases in oxygen consumption and body temperature, consistent with orexin bioactivities, suggesting that NERP-2 lies upstream of the orexin system.

The cell surface receptors or target proteins for NERPs have not yet been identified. There likely is a receptor specific for NERP-2 given that NERP-2, not NERP-1, activated the orexin system. NERP-2 provides a clue to our understanding of the complicated and well-organized mechanisms that regulate orexin neurons. This study exemplifies the combination of neuroanatomic and functional approaches to probing the physiological roles of NERP-2. The identification of NERP-2 as a peptide involved in the hypothalamic regulation of feeding provides important information about the neuronal network underlying the regulation of energy homeostasis.

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