Neuromedin U has a physiological role in the regulation of food intake and partially mediates the effects of leptin

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Jethwa, Preeti H., Caroline J. Small, Kirsty L. Smith, Asha Seth, Sarah J. Darch, Caroline R. Abbott, Kevin G. Murphy, Jeannie F. Todd, Mohammad A. Ghatei, and Stephen R. Bloom. Neuromedin U has a physiological role in the regulation of food intake and partially mediates the effects of leptin. Am J Physiol Endocrinol Metab 289: E301–E305, 2005. First published March 22, 2005; doi:10.1152/ajpendo.00404.2004.—Intracerebroventricular (ICV) administration of Neuromedin U (NMU), a hypothalamic neuropeptide, or leptin, an adipostat hormone released from adipose tissue, reduces food intake and increases energy expenditure. Leptin stimulates the release of NMU in vitro, and NMU expression is reduced in models of low or absent leptin. We investigated the role of NMU in mediating leptin-induced satiety. ICV administration of anti-NMU immunoglobulin G (IgG) (5 nmol) to satiated rats significantly increased food intake 4 h after injection, an effect seen for <8 h after injection. ICV administration of NMU (1 nmol) to fasted rats reduced food intake 1 h after injection compared with control, an effect attenuated by pretreatment with anti-NMU IgG. ICV administration of leptin (0.625 nmol) reduced 24-h food intake. This was partially attenuated by the administration of anti-NMU IgG [24 h after onset of dark phase: vehicle, 22.5 ± 2.0 g; leptin, 13.7 ± 2.3 g (P < 0.005 vs. vehicle); leptin/NMU IgG, 19.4 ± 1.3 g (P < 0.05 vs. leptin)]. Intraperitoneal administration of leptin (1.1 mg/kg body wt) reduced 24-h food intake. This was partially attenuated by ICV administration of anti-NMU IgG [24 h after onset of dark phase: vehicle, 31.4 ± 4.9 g; leptin, 20.8 ± 2.6 g (P < 0.01 vs. vehicle); leptin/NMU IgG, 28.7 ± 1.1 g (P < 0.01 vs. leptin)]. These results suggest that NMU plays a physiological role in the regulation of appetite and partially mediates the leptin-induced satiety.

intracerebroventricular; immunoblockade; anti-neuromedin U immunoglobulin G; antibody

THE HYPOTHALAMUS is the main central nervous system (CNS) region regulating feeding behavior and thermogenesis and thus controlling energy balance. Leptin is released into the circulation from adipose tissue and regulates food intake and energy expenditure via the hypothalamus (18, 22). Leptin influences hypothalamic neurons via the long-variant isoform of the leptin receptor Ob-Rb (3, 18, 20, 22).

Neuromedin U (NMU) is a hypothalamic neuropeptide implicated in the control of energy balance (12, 13, 28). Intracerebroventricular (ICV) or intraparaventricular administration of NMU inhibits feeding and increases energy expenditure (12, 13, 28). These effects are thought to be mediated by the hypothalamic NMU2 receptor, expression of which is concentrated in the ependymal layer of the third ventricle, the paraventricular nucleus (10, 12, 19, 27), and the arcuate nucleus (10). NMU mRNA is predominantly expressed in the arcuate nucleus, the suprachiasmatic nucleus, and the dorsomedial nucleus (10, 12). These nuclei also express the Ob-Rb (15).

Recently, it has been shown that NMU mRNA expression in the arcuate nucleus is reduced following a 48-h fast (when leptin levels are low) and in leptin-deficient ob/ob mice (12). Wren et al. (28) reported an increase in NMU release from basal hypothalamic explants following incubation with leptin in vitro. However, there have been few other published data on the interaction between leptin and NMU systems.

We have investigated the role of endogenous and exogenous NMU in the hypothalamus by blocking its effects on food intake by use of ICV administration of anti-NMU immunoglobulin G (IgG). In addition, we have used this antibody to investigate whether NMU mediates the anorectic effect of leptin.

MATERIALS AND METHODS

Animals. Male Wistar rats (specific pathogen free; Charles River, Margate, UK), weighing 200–250 g, were maintained in individual cages under controlled temperature (21–23°C) and light (12:12-h light-dark cycle, lights on at 0700) with ad libitum access to food (RM1 Diet; SDS UK, Witham, UK) and water, unless otherwise described. Food intake and body weight were measured daily throughout the study, and all rats were handled daily to habituate them and minimize any stress. All animal experimentation was conducted in accordance with accepted standards of humane animal care and carried out under the British Home Office 1986 Animals (Scientific Procedures) Act, license no. 70/5516.

Materials. Full-length rat NMU (NMU-23) was custom synthesized (IAF Biochem, Quebec, ON, Canada) and used in all in vitro and in vivo experiments. Recombinant murine leptin was a gift from M. Chiesi and N. Levens from Novartis (Basel, Switzerland). Reagents for the hypothalamic explant experiments were supplied by BDH (Poole, Dorset, UK). All peptides were reconstituted at the beginning of each study in vehicle (nonimmune rabbit serum).

Antibody purification. The NMU IgG was produced in New Zealand white rabbits after immunization with synthetic rat NMU (Cambridge Research Biochemicals, Cleveland, UK) conjugated to BSA by glutaraldehyde and emulsified in complete Freund’s adjuvant. Ten weeks later, the rabbits received a booster injection using NMU conjugated in the same manner but emulsified in incomplete Freund’s adjuvant. The antisera used in these studies was obtained 1 wk after the booster injection, as previously described (6, 7, 25). Cross absorption carried out with both molecular forms of NMU and with other peptides known to have similar structures showed that anti-NMU IgG cross-reacts 100% with both molecular forms of NMU, the icosapeptide (NMU-23) and an octapeptide (NMU-8), which is identical to the COOH terminus of NMU-23 (16). However, NMU-IgG does not cross-react with adrenocorticotropic hormone, proopiomelanocortin(α₁-76), corticotropin-like intermediate lobe peptide, β-lipotrophin,
Ad libitum-fed male Wistar rats received a single 5-μl ICV injection of saline, vehicle (NIRS), anti-NMU IgG (5 nmol), CW8 IgG (5 nmol), CY8 IgG (5 nmol), or α-melanocyte-stimulating hormone (α-MSH, 0.5 nmol; positive control) (n = 6–7/group), in the early light phase. Food was reweighed 1, 2, and 4 h after injection, as detailed above.

IVC administration of pretreatment of anti-NMU IgG on NMU-induced anorexia. Male Wistar rats fasted for 24 h (n = 10–12/group) received a 2-μl ICV injection in the early light phase of either NMU IgG (5 nmol) or vehicle. Sixty minutes later, the rats were injected with either NMU (1 nmol) or vehicle (2 μl volume). The injection regimen for the four groups was as follows: vehicle/vehicle, vehicle/NMU, NMU IgG/vehicle, or NMU IgG/NMU. The 1 nmol dose of NMU was the lowest dose to significantly reduce food intake when injected ICV in previous studies (28). The 60-min time point was chosen because a significant increase in food intake was observed within 1 and 2 h after injection with anti-NMU IgG in the previous study, thus suggesting that the antibody was active at 1–2 h after injection. Food was presented after the second ICV injection and reweighed at 1, 2, 4, 8, and 24 h after second injection.

IVC administration of anti-NMU IgG on leptin-induced anorexia. Male Wistar rats fasted for 24 h (n = 10–12/group) received a 5-μl ICV injection of either vehicle or leptin (0.625 nmol) in the early light phase. At the onset of the dark phase, rats received another 5-μl ICV injection of either vehicle or NMU IgG (5 nmol). The injection regimen for the four groups was as follows: vehicle/vehicle, leptin/vehicle, vehicle/NMU IgG, or leptin/NMU IgG. The 0.625 nmol (10-μg) dose of leptin has previously been shown to reduce food intake following ICV administration (9). Food was reweighed at 1, 2, 4, and 24 h after second injection.

IVC administration of anti-NMU IgG on peripheral leptin-induced anorexia. Another group of male Wistar rats was fasted for 24 h (n = 8–10/group) and given an intraperitoneal injection (0.5 ml) of either vehicle or leptin (1.1 mg/kg body wt) in the early light phase. At the onset of the dark phase, rats received a 5-μl ICV injection of either vehicle or NMU IgG (5 nmol). The injection regimen for the four groups was as follows: vehicle/vehicle, leptin/vehicle, vehicle/NMU IgG, or leptin/NMU IgG. The 1.1 mg/kg body wt dose of leptin has previously been shown to alter hypothalamic factors such as AMP kinase (1). Food was reweighed at 1, 2, 4, and 24 h after second injection.

Statistics. Data are presented as means ± SE. For all IVC studies, groups were compared by one-way ANOVA followed by a post hoc Fisher’s least significant difference test (Systat, Evanston, IL). In all cases, P < 0.05 was considered statistically significant.

RESULTS

IVC administration of anti-NMU IgG on food intake in satiated rats. NMU IgG (5 nmol) had no effect on food intake 1 h after injection compared with vehicle or saline (0–1 h after injection: saline, 1.8 ± 0.7 g; vehicle, 1.9 ± 0.2 g; NMU IgG, 2.5 ± 0.7 g [P = not significant (NS) vs. saline or vehicle]; n = 10–12/group). NMU IgG increased food intake compared with saline and vehicle controls 2 h after injection, but failed to reach statistical significance [0–2 h after injection: saline, 2.8 ± 0.9 g; vehicle, 2.6 ± 0.9 g; NMU IgG, 4.9 ± 1.1 g (P = 0.08 vs. saline and vehicle)]. However, NMU IgG significantly increased food intake compared with vehicle and saline controls 4 h after injection [0–4 h after injection: saline, 3.2 ± 1.0 g; vehicle, 3.0 ± 0.2 g; NMU IgG, 6.2 ± 1.0 g (P < 0.01 vs. vehicle and saline); Fig. 1]. Cumulative food intake remained significantly elevated up to 8 h after injection compared with vehicle and saline controls [0–8 h after injection: saline, 4.2 ± 1.3 g; vehicle, 4.1 ± 0.5 g; NMU IgG, 8.7 ± 1.2 g (P < 0.01 vs. vehicle and saline); Fig. 1]. There was no
significant difference between the saline and the vehicle group at any given time point.

**ICV administration of pretreatment of anti-NMU IgG on NMU-induced anorexia.** ICV administration of NMU (1 nmol) significantly reduced food intake 1 h after injection compared with vehicle controls. During this period, the NMU IgG had no effect on food intake compared with saline-treated controls. The reduction in food intake caused by NMU was attenuated with vehicle controls. During this period, the NMU IgG had no significantly reduced food intake 1 h after injection compared NMU-induced anorexia.

Light phase food intake was reduced to a similar extent in both leptin-pretreated groups before the second injection, although this failed to reach statistical significance [light phase food intake: vehicle/vehicle, 15.0 ± 2.1 g; leptin/vehicle, 13.7 ± 0.8 g; leptin/NMU IgG, 14.1 ± 1.8 g (n = 8–10/group); data not shown]. There was a significant 33% reduction in 24-h food intake in the leptin/vehicle group (P < 0.01 vs. vehicle/vehicle; Fig. 4D). This decrease was partially attenuated by the administration of anti-NMU IgG [0-24 h food intake: vehicle/vehicle, 31.4 ± 4.9 g; leptin/vehicle, 20.8 ± 2.6 g (P < 0.01 vs. vehicle/vehicle); leptin/NMU IgG, 28.7 ± 1.1 g (P = NS vs. vehicle/vehicle; P < 0.01 vs. leptin/vehicle); Fig. 4D]. No significant difference was observed at any other time point (Fig. 4).

**DISCUSSION**

We (28) have reported previously that CNS administration of NMU reduced food intake, increased locomotor activity and grooming behavior, and stimulated the hypothalamic-pituitary-adrenal (HPA) axis. Others have reported that ICV administration of NMU increases energy expenditure (12, 13). To investigate the role of endogenous NMU in energy homeostasis, we examined the effect of NMU on food intake by blocking endogenous and exogenous NMU by use of anti-NMU IgG. ICV administration of anti-NMU IgG to satiated rats caused a significant increase in cumulative food intake 4 and 8 h after injection. These findings are in agreement with Kojima et al. (14), who showed ICV administration of NMU antiserum to increase food intake in freely feeding male Wistar rats at 12 h after injection. To investigate the specificity of the antibody, we investigated the effects of a specific anti-NMU IgG on the anorectic effects of exogenous NMU. Preadministration of anti-NMU IgG attenuated the anorectic effects of NMU following ICV administration. The effects of central...
anti-NMU IgG suggest that NMU plays a physiological role in the regulation of appetite.

ICV administration of leptin reduces food intake, activates the sympathetic nervous system, and increases energy expenditure (5, 8, 23). These effects are similar to those observed following ICV administration of NMU (4, 12, 13, 28). Wren et al. (28) proposed that NMU release might be stimulated by leptin and that NMU synthesis might be inhibited in its absence.
sence. However, there are few data on the interaction between the leptin and NMU signaling systems in the regulation of energy balance. We have shown that leptin reduces food intake 24 h after the onset of the dark phase following both ICV and peripheral administration. ICV administration of NMU-IgG partially attenuates the anorectic effect of both ICV and peripheral leptin. It is unknown whether the effect of leptin on NMU signaling is direct or is mediated via an indirect neuronal pathway. It would be interesting to investigate whether hypothalamic NMU neurons express Ob-Rb and whether leptin acts at these NMU neurons. Hanada et al. (11) recently reported that NMU-null mice are obese due to hyperphagia and have reduced locomotor activity and decreased energy expenditure. ICV administration of leptin to these mice reduced body weight, implying that NMU signaling is not vital for the anorexigenic action of leptin (11). A number of neurotransmitters are known to mediate hypothalamic leptin signaling (2, 22, 24), and these other systems may compensate for the absence of NMU signaling in NMU-null mice.

In summary we have shown that immunoblockade of endogenous NMU signaling increases food intake, suggesting that NMU has a physiological role in the regulation of food intake. NMU may have a role in mediating the effect of leptin on food intake.

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