Peripheral Administration of Nesfatin-1 Reduces Food Intake in Mice: The Leptin-Independent Mechanism

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Nesfatin-1 is a novel satiety molecule in the hypothalamus and is also present in peripheral tissues. Here we sought to identify the active segment of nesfatin-1 and to determine the mechanisms of its action after peripheral administration in mice. Intraperitoneal injection of nesfatin-1 suppressed food intake in a dose-dependent manner. Nesfatin-1 has three distinct segments; we tested the effect of each segment on food intake. Injection of the midsegment decreased food intake under leptin-resistant conditions such as db/db mice and mice fed a high-fat diet. After injection of the midsegment, expression of c-Fos was significantly activated in the brainstem nucleus tractus solitarius (NTS) but not in the hypothalamic arcuate nucleus; the nicotinic cholinergic pathway to the NTS contributed to midsegment-induced anorexia. Midsegment injection significantly increased expression of proopiomelanocortin and cocaine- and amphetamine-regulated transcript genes in the NTS but not in the arcuate nucleus. Investigation of mutant midsegments demonstrated that a region with amino acid sequence similarity to the active site of agouti-related peptide was indispensable for anorexigenic induction. Our findings indicate that the midsegment of nesfatin-1 causes anorexia, possibly by activating POMC and CART neurons in the NTS via a leptin-independent mechanism after peripheral stimulation. (***Endocrinology*** **150: 662–671, 2009)**

The central nervous system (CNS) contains a number of neuroactive molecules that regulate energy homeostasis; impaired regulation of energy homeostasis can result in obesity due to increased appetite and decreased energy expenditure. Appetite-regulating molecules in the CNS are categorized as either anorexigenic or orexigenic depending on their function. Among anorexigenic molecules, α-MSH, which is posttranslationally processed from proopiomelanocortin (POMC), possesses a marked anorexigenic activity, and functions as a key molecule in the up- or downstream signaling mediated by other satiety molecules in the CNS (1, 2). Peripheral signaling is tightly linked to molecules in the CNS that regulate feeding behavior. Neurons expressing POMC in the CNS are restricted to the hypothalamic arcuate nucleus (Arc) and brainstem nucleus tractus solitarius (NTS), which differently receive and integrate varying peripheral signals with respect to appetite regulation (1–3). These differences are seen in the activation of the CNS by the potent anorexigenic molecules leptin and cholecystokinin (CCK). Leptin originates in peripheral adipose tissue and reaches its specific receptor in the Arc via a humoral system to activate POMC neurons (4, 5), whereas CCK is derived from the peripheral gastrointestinal tract and activates POMC neurons in the NTS via the vagal afferent nerve system (6, 7). In addition to stimulating POMC neurons, these peripheral signals may exert simultaneous influences on other appetite-regulating molecules in the CNS. Thus, peripheral stimulation by leptin and CCK activates neurons expressing CART (8, 9), an anorexigenic molecule located in many brain areas including the Arc and NTS.

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Abbreviations: AgRP, Agouti-related peptide; Arc, arcuate nucleus; bw, body weight; CART, cocaine- and amphetamine-regulated transcript; CCK, cholecystokinin; CNS, central nervous system; MC3R, melanocortin 3 receptor; NTS, nucleus tractus solitarius; POMC, proopiomelanocortin.

Because human obesity shows a conditioned resistance to leptin, recognition of bioactive molecules that exert anorexia independent of leptin signaling is important (10–12). Previously, we demonstrated that nesfatin-1 in the hypothalamus has a robust anorexigenic activity after intracerebroventricular administration and that intracerebroventricular injection of an antibody neutralizing endogenous nesfatin-1 significantly stimulates food intake (13). It is clear that the central anorexigenic function induced by nesfatin-1 is independent of leptin signaling in the hypothalamus, because nesfatin-1-induced anorexia occurs in rats with a leptin receptor mutation. Nesfatin-1 is distributed not only throughout the brain (13–15) but also in peripheral tissues, such as adipose tissue (13). Recent observations have shown that most peripherally injected nesfatin-1 crosses the blood-brain barrier to reach brain tissue (16, 17). However, there are no reports on the effects of peripheral administration of nesfatin-1. Nor has the functionally active domain of nesfatin-1 been identified; such identification would be invaluable to future development of nesfatin-1 analogs as antiobesity drugs, because its specific receptor remains to be elucidated.

The present study assessed the effects of peripherally administered nesfatin-1 on food intake. We also identified the active segment of nesfatin-1 and investigated whether leptin-dependent signaling is involved in the effect of peripheral nesfatin-1 on feeding regulation in mice.

Materials and Methods

Substances

A cDNA encoding mouse nesfatin-1 fused with glutathione S-transferase, and a His-tag was constructed in *Escherichia coli* BL21(DE3) transfected into the pET41a(+) expression vector. Proteins were purified using Ni-nitro-triacetic acid-agarose and glutathione-agarose beads, released by thrombin digestion, and further purified by reverse-phased HPLC (C18, 4.6 × 250 mm). Amino acid sequence was confirmed by both mass spectrum analysis and N-terminal amino acid sequence analysis. Human or rat nesfatin-1, three segmental peptides of mouse nesfatin-1, and mutant peptide midsegments were synthesized by conventional methods of peptide chemistry; each peptide was confirmed by HPLC and mass spectrum analysis. Mouse leptin was purchased from R&D Systems Inc. (Minneapolis, MN).

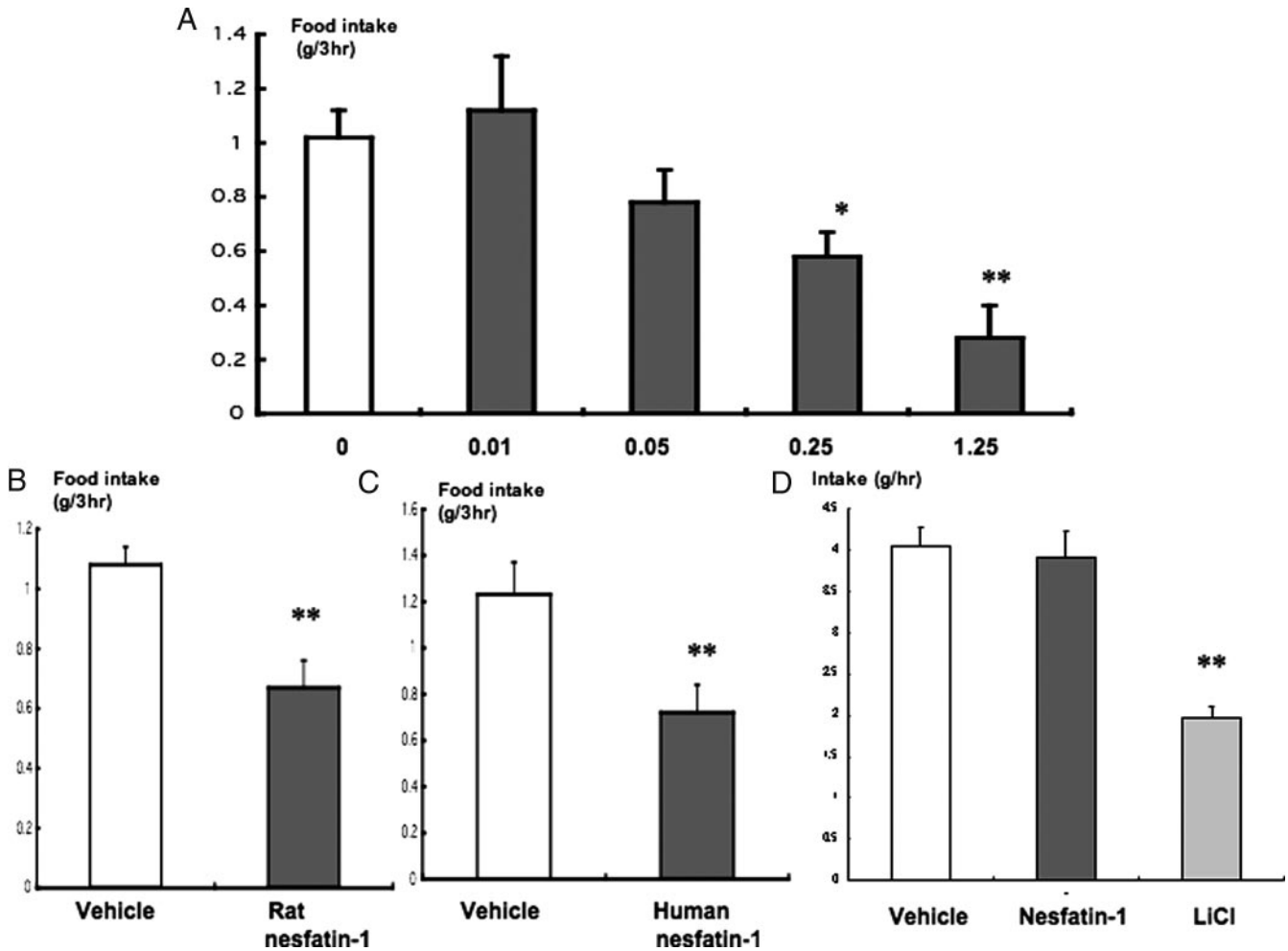

FIG. 1. Effects of nesfatin-1 injection on food intake. Mice were given an ip injection of varying doses (nmol/g bw) of mouse nesfatin-1 (A) or 0.25 nmol/g bw of rat (B) and human nesfatin-1 (C). Food intake for 3 h was estimated. In the taste aversion assay (D), mice were given an ip injection of nesfatin-1 or LiCl, and the 1-h consumption of saccharin solution was estimated. The groups consisted of five to six mice (A), five to nine mice (B and C), or five mice (D). Data are expressed as means ± SEM. *, $P < 0.05$; **, $P < 0.01$ *vs.* vehicle or no injection (Student's *t* test in B and C).

Animals

Procedures for animal care and use in these studies were approved by the Review Committee on Animal Use at Gunma University (Maebashi, Japan). Adult male ICR mice were individually maintained on a 12-h light, 12-h dark schedule (lights on at 0600 and off at 1800 h) and provided with *ad libitum* access to laboratory chow and tap water. Experiments involving ip bolus injection were started 30 min before the beginning of the dark cycle. db/db mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Adult male ICR mice were fed either a regular diet (6.4% fat) or a high-fat diet containing 45% fat for 30 d, as described previously (18). Food intake was measured to the nearest 0.1 g.

To investigate the possible involvement of the cholinergic and adrenergic systems, we examined changes in the anorexigenic effects of the midsegment fragment of nesfatin-1 after pretreating the mice with the appropriate blocking drugs. Atropine methyl nitrate [2 mg/kg body weight (bw)], nicotinic hexamethonium (10 mg/kg bw), or bretylium tosylate (15 mg/kg bw) was ip administered 15 min before the injection of the midsegment fragment of nesfatin-1.

Assay for conditioned taste aversion

The aversive effects of nesfatin-1 were examined in mice as described previously (19, 20). Mice were conditioned to access water from two bottles for 2 h each day for 3 d. On d 4, the mice were given 0.15% sodium saccharin instead of water in one bottle for the 2-h period. Before access to drinking water on d 5, the mice received an ip injection of saline, 0.25 nmol/g bw mouse nesfatin-1, or 0.15 M LiCl at 2% of bw. The consumption of saccharin solution (in grams) over 1 h by each mouse was estimated.

Detection of expression of c-Fos-like cells and appetite-regulating genes in Arc and NTS

Mice were injected ip with 0.5 nmol/g bw of the midsegment fragment 2 h before lights off. The mice were starved for 2 h and then killed. Brains were fixed in 4% paraformaldehyde and then transferred to 30% sucrose. Expression of c-fos-like immunoreactive cells was detected as described previously (15). Floating sections (40 μm thick) were rinsed in PBS and then incubated with 0.3% H_2O_2 for 20 min. After rinsing, sections were blocked for 30 min and incubated with rabbit anti-c-Fos rabbit antibody (Ab-5, 1:100,000; Calbiochem, La Jolla, CA) overnight at room temperature. The sections were then rinsed and incubated with biotinylated goat antirabbit IgG for 30 min. After washing, sections were incubated with ABC reagent for 30 min (Vector Laboratories, Burlingame, CA; 1:500). The sections were washed with PBS and 0.175 M sodium acetate buffer (pH 5.6), and color staining was developed using a nickel-diaminobenzidine solution (10 g/liter nickel ammonium sulfate, 0.2 g/liter diaminobenzidine and 0.006% H_2O_2 in sodium acetate buffer) for 5 min.

Discrete regions of the Arc and NTS were dissected, and total RNAs were extracted from each tissue using Trizol reagent (Invitrogen). Real-time quantitative RT-PCR using SYBG premix Ex *Taq* II probes (Takara Bio,

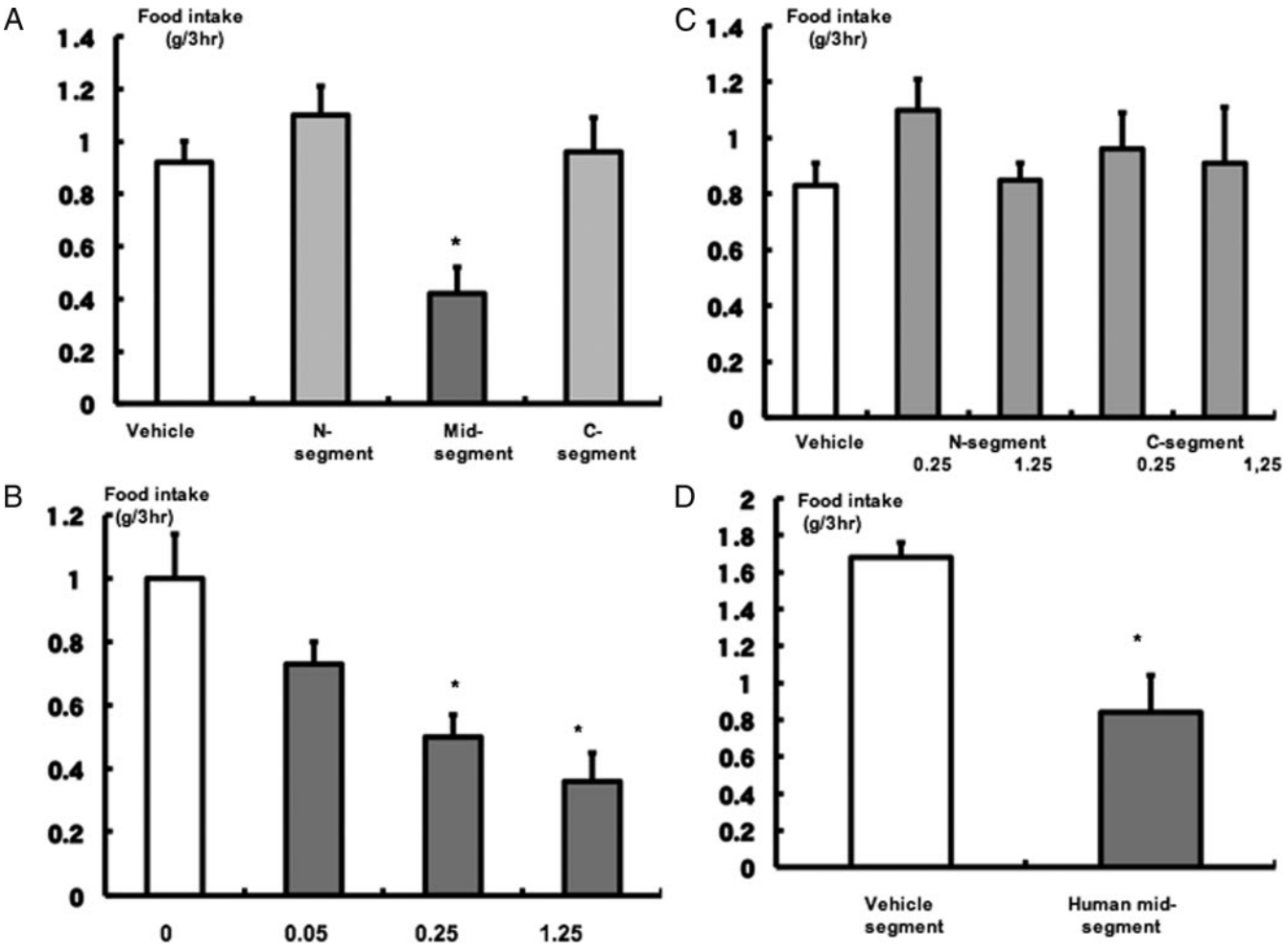

FIG. 2. Effect of different segments of nesfatin-1 on food intake. Mice were given a 0.25 nmol/g bw ip injection of a mouse nesfatin-1 segment (A), or varying doses (nmol/g bw) of the mouse midsegment (B), mouse N- or C-segments (C), or human midsegment (0.25 nmol/g bw, D). Food intake for 3 h was determined. The groups consisted of eight mice (A), seven to nine mice (B), five to 11 mice (C), or five mice (D). Data are expressed as means ± SEM. *, $P < 0.01$ *vs.* vehicle or no injection (Student's *t* test in D).

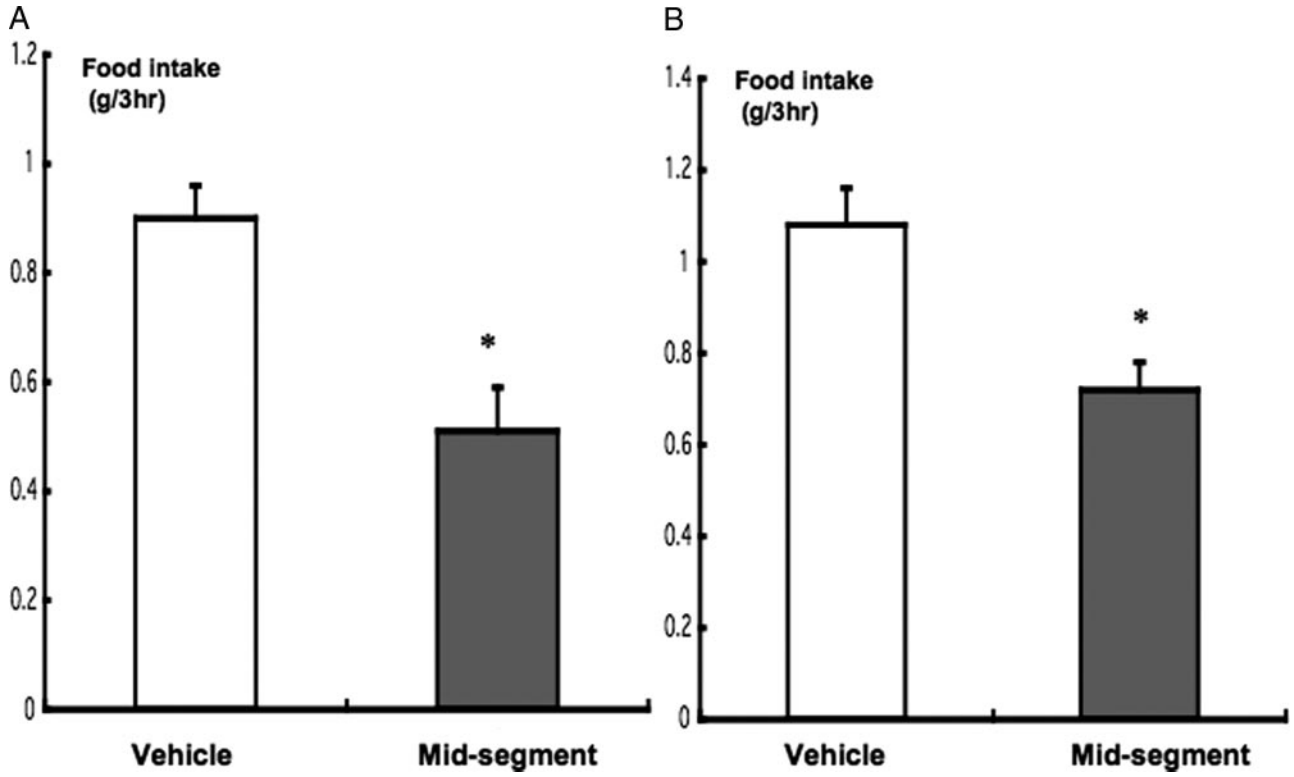

FIG. 3. Effect of midsegment injection on food intake in db/db obese mice. Lean control (A) and db/db (B) mice were given a 0.25 nmol/g bw ip injection of the mouse midsegment, and food intake for 3 h was determined. Each group consisted of five to eight mice. Data are expressed as means ± SEM. *, $P < 0.01$ *vs.* vehicle injection (Student's *t* test).

Inc., Shiga, Japan) was employed to measure the levels of expression of appetite-regulating genes. The following sets of primers were used: POMC, 5′-CATTAGGCTTGGAGCAGGTC-3′ and 5′-TCTTGATGATGGCGTTCTTG-3′; CART, 5′-TGGATGATGCGTCCCACGAG-3′ and 5′-CGGAATGCGTTTACTCTTGAGC-3′; AgRP, 5′-GGTGCTAGATCCACAGAACCG-3′ and 5′-CCAAGCAGGACTCGTGCAG-3′; and GAPDH,

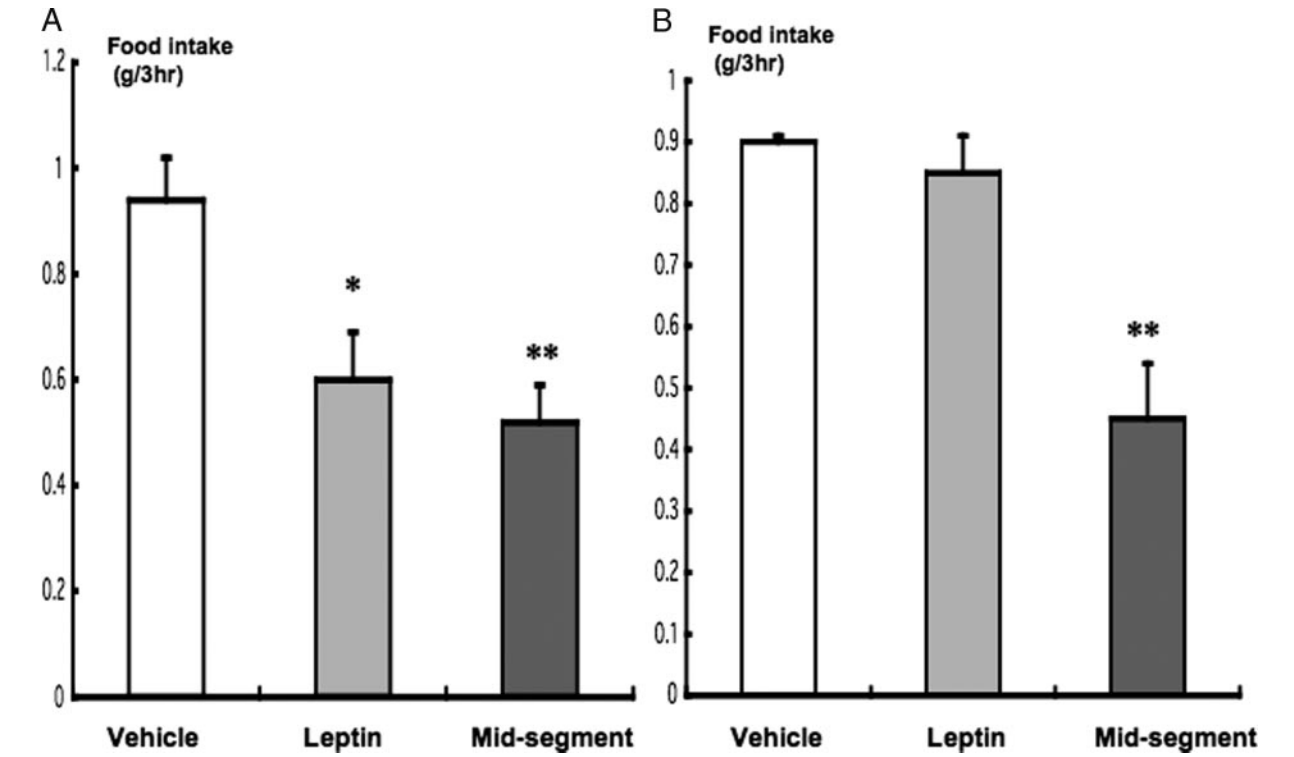

FIG. 4. Effect of midsegment injection on food intake in mice fed a high-fat diet. Mice fed either a regular diet (A) or a high-fat diet (B) were given an ip injection of vehicle, leptin (0.25 nmol/g bw), or the midsegment (0.25 nmol/g bw). Food intake for 3 h was determined. The groups consisted of five to seven mice (A) or eight to nine mice (B). Data are expressed as means ± SEM. *, $P < 0.05$; **, $P < 0.01$ *vs.* vehicle injection.

5′-GGCACAGTCAAGGCTGAGAATG-3′ and 5′-ATGGTGGTGAAGACGCCAGTA-3′.

Data analysis

All data are shown as means ± SEM. Statistically significant differences between the means were identified using ANOVA, unless otherwise indicated.

Results

Changes in food intake after peripheral injection of nesfatin-1

Figure 1A shows the effect of ip injection of varying doses of mouse nesfatin-1 on food intake in mice. Nesfatin-1 injection decreased food intake in a dose-dependent manner; the IC_{50} of nesfatin-1 was estimated to be approximately 0.3 nmol/g bw, a dose nearly equivalent to the previously estimated IC_{50} values of 0.4 nmol/g bw for an α-MSH analog (21) and 0.13∼0.31 nmol/g bw for leptin (22–24). An ip injection of 0.25 nmol/g bw of rat or human nesfatin-1 also suppressed food intake in mice; the potency of these peptides was similar to that induced by mouse nesfatin-1 (Fig. 1, B and C).

Nesfatin-1 influence on conditioned taste aversion

Because peripheral injection of some anorexigenic molecules has been shown to produce aversive effects that result in reduced appetite (19), we examined this aspect of nesfatin-1 in mice. In a conditioned taste aversion assay (Fig. 1D), the consumption of saccharin solution was unaltered by mouse nesfatin-1 injection (4.04 ± 0.23 g) but was significantly suppressed by injection of LiCl (1.96 ± 0.15 g), a toxic substance that causes saccharin avoidance (25). These data agree with previous reports that endogenous anorexigenic molecules that act in the hypothalamus, including neuromedin U and glucagon-like peptide-1, do not affect conditioned taste aversion (20, 26).

Identification of the functional site in nesfatin-1 for reduced food intake

To identify the sites in nesfatin-1 responsible for reducing food intake, we synthesized three peptides that met the following

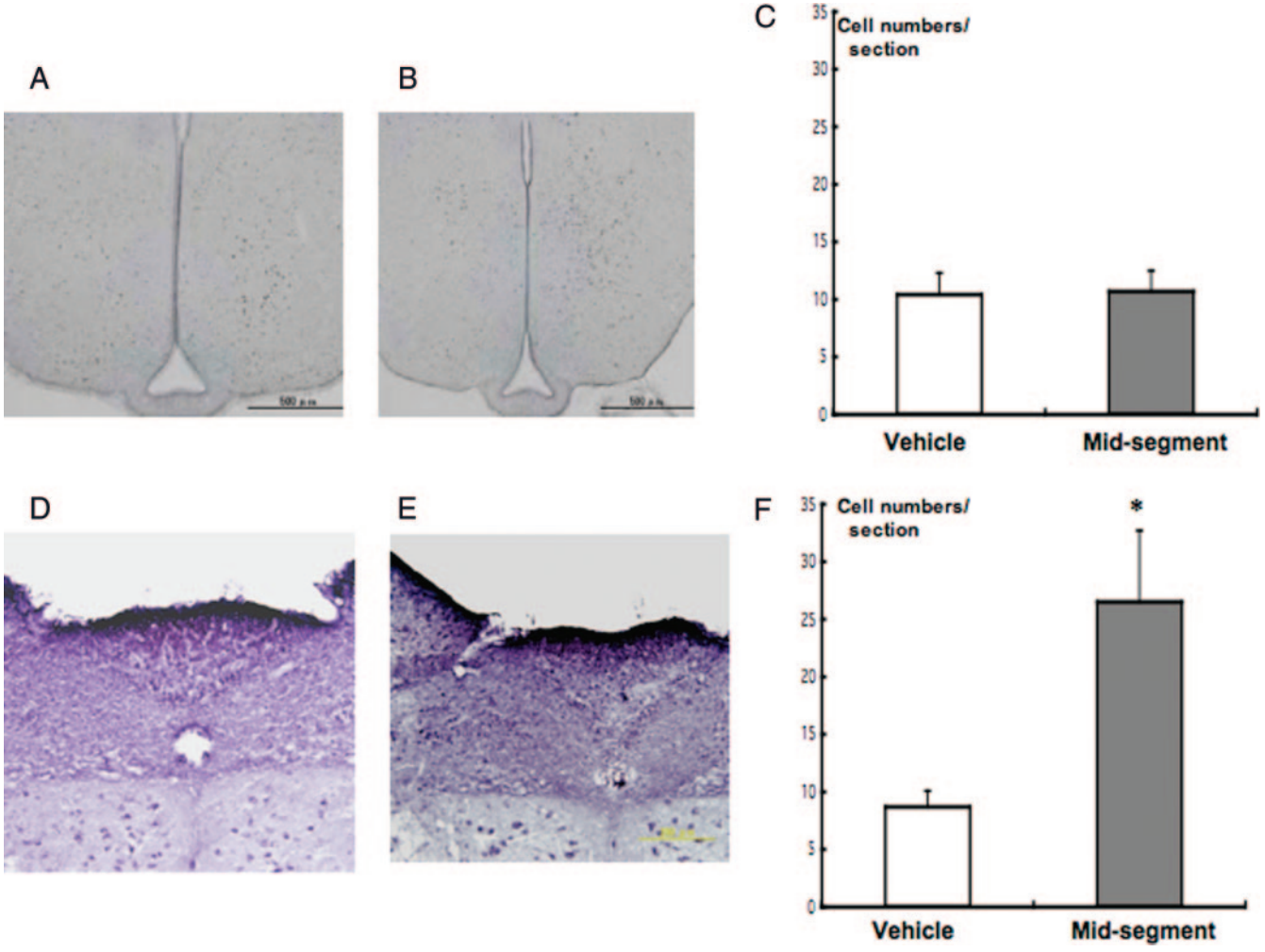

FIG. 5. Effect of midsegment injection on c-Fos expression in ARC and NTS. Mice were given an ip injection of vehicle or the midsegment, and c-Fos-immunopositive neurons were identified in the Arc and NTS. A (vehicle) and B (midsegment) show representative examples of c-Fos expression in the Arc; D (vehicle) and E (midsegment) show representative examples of c-Fos-positive cells in the NTS area. C and F, Numbers of c-Fos-positive cells in the Arc and NTS area, respectively. Each group consisted of four to six mice. Data are expressed as means ± SEM. *, $P < 0.05$ *vs.* vehicle injection (Student's *t* test).

two conditions. The first condition was based on the recognition of a decision constant for the α-helix of nesfatin-1 estimated by prediction of protein secondary structure using the Parallel Protein Information system (http://mbs.cbrc.jp/papia/papiaJ.html, Chou-Fasman and New Joint methods). The second condition was determined by the recognition of the secure turn segments of nesfatin-1 by prediction of protein secondary structure as described previously (27). Consequently, three peptide segments were identified: an N-terminal segment (23 amino acids), a midsegment (30 amino acids), and a C-terminal segment (29 amino acids). These segments correspond to amino acid residues 1–23, 24–53, and 54–82 of mouse nesfatin-1, respectively. It is unclear whether these segments actually represent endogenous fragments that can be degraded from nesfatin-1.

The effect of ip injection of each of the three segments on food intake was studied in mice. Injection of the midsegment caused a dose-dependent decrease in food intake (Fig. 2); the IC_{50} was estimated to be approximately 0.36 nmol/g bw, similar to that of nesfatin-1 (0.35 nmol/g bw). In contrast, neither the N- nor C-terminal segments significantly affected feeding even using a higher dose of each peptide (1.25 nmol/g bw).

Changes in food intake after injection of a midsegment derived from other species

The amino acid sequence in the midsegment of nesfatin-1 is highly conserved among different species. The amino acid sequences of mouse and rat are identical, and only two amino acid residues differ between human and mouse. We examined the activity of the human nesfatin-1 midsegment in mice. An ip injection of the human midsegment caused a significant decrease in food intake (1.68 ± 0.08 *vs.* 0.84 ± 0.20 g) compared with mice that received the vehicle only (Fig. 2).

Effects of the midsegment on food intake in db/db mice

As the central function of nesfatin-1 is not blocked in Zucker obese rats, which have a leptin receptor mutation (13), we next studied peripheral function induced by the nesfatin-1 midsegment in db/db obese mice, which similarly have a mutation of the leptin receptor (28). Figure 3 shows the effects of ip injection of the midsegment on food intake in lean control (bw, 26.4 ± 0.3 g) and db/db obese mice (bw, 51.3 ± 0.3 g). The midsegment caused a significant decrease in food intake in obese mice (1.08 ± 0.08 *vs.* 0.72 ± 0.06 g) compared with vehicle controls, and the magnitude of this reduction was similar to that observed in lean mice.

Effects of the midsegment on food intake in mice on a high-fat diet

Because rodents fed a high-fat diet exhibit leptin resistance that is considered to offer an animal model of human obesity (18, 29, 30), we examined the effects of midsegment injection on food intake in leptin-resistant mice. Intraperitoneal injection of leptin suppressed the food intake of mice fed a regular diet (bw, 37.9 ± 0.5 g), but not of mice fed a high-fat diet (bw, 46.6 ± 1.3 g) (Fig. 4). However, these leptin-resistant mice did show a significantly

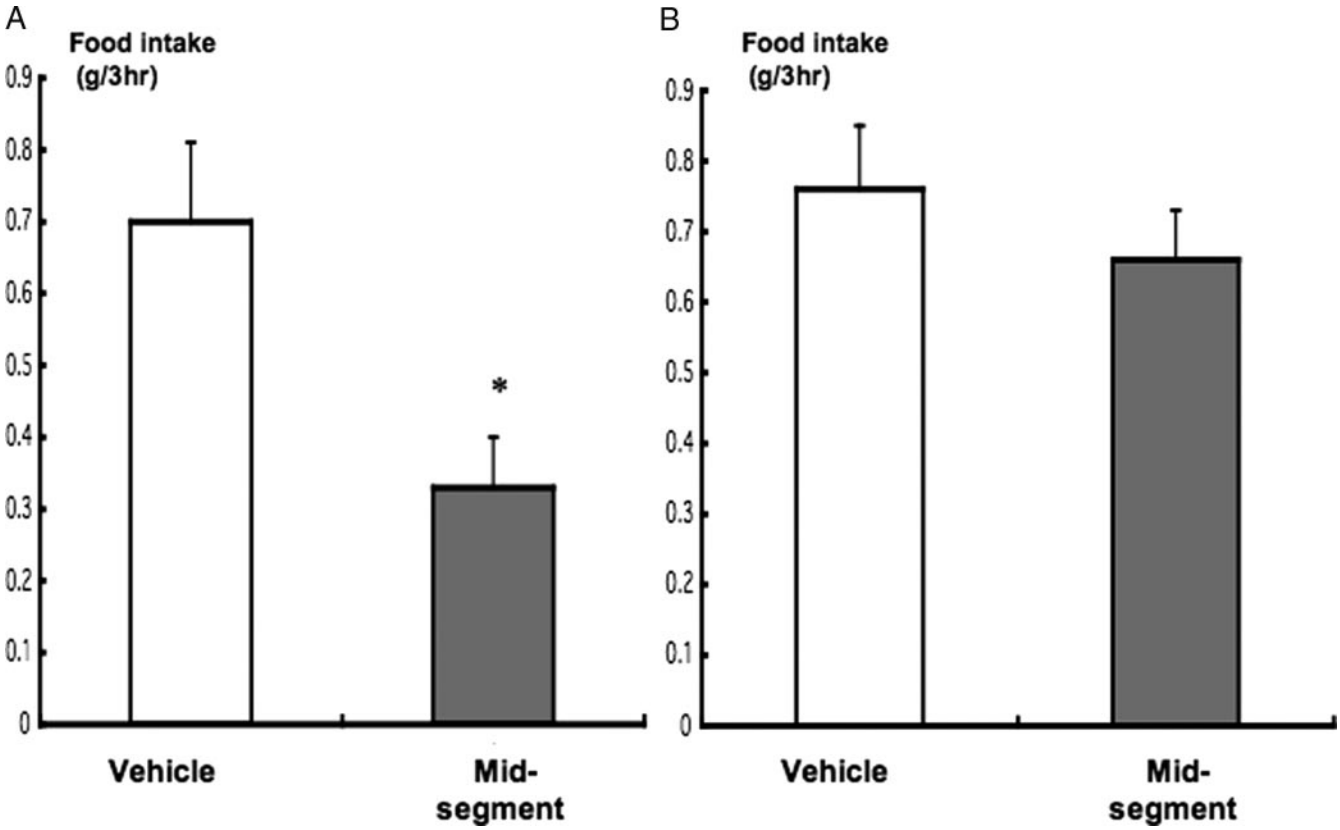

FIG. 6. Effects of blockers for the cholinergic pathways on midsegment-induced anorexia. Mice were given an ip injection of atropine methyl nitrate (2 mg/kg bw, A) or nicotinic hexamethonium (10 mg/kg bw, B). After 15 min, the mice received an ip injection of vehicle or the midsegment (0.25 nmol/g bw). Food intake for 3 h was determined. Each group consisted of five to seven mice. Data are expressed as means ± SEM. *, $P < 0.05$ *vs.* vehicle injection (Student's *t* test).

suppressed food intake (0.90 ± 0.01 *vs.* 0.45 ± 0.09 g) after injection of the midsegment compared with vehicle controls.

Effects of midsegment on c-Fos expression in Arc and NTS

Arc and NTS neurons receive abundant but different stimulation from the periphery for regulation of energy homeostasis (1, 2). In addition, blood-borne peptides sometimes act on the neurons in the area postrema, which lacks a blood-brain barrier. We examined the effects of ip injection of the midsegment on the expression of c-Fos, an indicator of neuronal activity, in the hypothalamus and brainstem of mice. The midsegment did not induce c-Fos expression in neurons in the Arc but significantly stimulated c-Fos expression in neurons in the NTS and area postrema (Fig. 5).

Effects of blockers of cholinergic pathways on midsegment-induced anorexia

Signal transmission from the vagal afferent nerves to the neurons in the NTS is known to involve neurons with muscarinic and nicotinic cholinergic fibers (31, 32). To characterize the possible role of afferent nervous systems to the NTS in the anorexia induced by the midsegment, we examined the influence of compounds that block each of these cholinergic pathways, as described previously (33). Whereas atropine methyl nitrate, an antagonist for the muscarinic cholinoceptor, did not affect midsegment suppression of food intake, the nicotinic cholinergic blocker hexamethonium abolished this suppression (Fig. 6).

Effects of midsegment on expression of POMC and CART mRNA in Arc and NTS

Neurons in the Arc and NTS produce the anorexigenic molecules POMC and cocaine- and amphetamine-regulated transcript (CART), and the levels of these molecules are influenced by peripheral signaling (5–9). We examined the effect of ip injection of the midsegment on these brain molecules in mice and found that it did not cause any significant changes in the expression of POMC, CART, or agouti-related peptide (AgRP) in the Arc (Fig. 7, A–C). In contrast, significant increases in expression of POMC and CART were observed in the NTS after ip injection of the midsegment.

Homology of the midsegment fragment with α-MSH and agouti-related peptide AgRP

To identify the essential core sites of the midsegment, we performed a homology search of the amino acid sequence of the human nesfatin-1 midsegment against those of other molecules known to regulate appetite. The C-terminal region of the midsegment contains an amino acid sequence H-F-R identical to that of α-MSH; this sequence has the characteristic motif of a melanocortin 3 receptor (MC3R) and MC4R recognition site (34, 35). The central region of the midsegment contains the amino acid sequence LKQVIDV that shows 42.9% identity and 85.7% similarity to an amino acid sequence in human AgRP; this sequence includes the site in AgRP involved in increased energy expenditure (36). The alignment of amino acids in the C-terminal and inner regions of the midsegment is well preserved in human, mouse, and rat nesfatin-1.

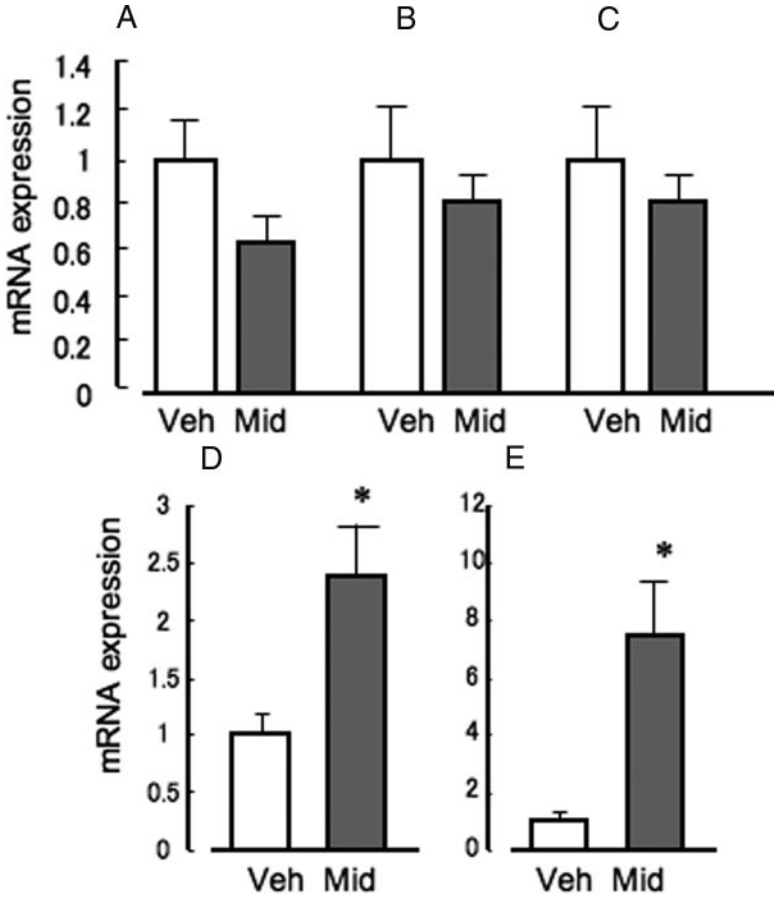

FIG. 7. Effects of midsegment injection on expression of appetite-regulating genes in the Arc and NTS. Mice were given an ip injection of vehicle (veh) or the midsegment (mid), and the brain Arc and NTS were then dissected. Gene expression was estimated by real-time quantitative RT-PCR. Each group consisted of four mice. Data are expressed as the means ± SEM of the ratios of expression of each gene against GAPDH. A, Arc-POMC; B, Arc-CART; C, Arc-AgRP; D, NTS-POMC; E, NTS-CART. *, $P < 0.05$ *vs.* vehicle injection (Student's *t* test).

Effects of mutant midsegments on food intake

To confirm that the region of the midsegment similar to α-MSH is important for anorexia, we synthesized two different mutant midsegments of nesfatin-1 (Fig. 8A). In the first mutant, the α-MSH-similar amino acid sequence was substituted with alanine residues; this mutant was designated M30 MSH-A. In the second mutant, the AgRP-similar amino acid sequence was substituted with alanine residues; this mutant was designated M30 Ag-A.

We examined changes in food intake in mice that had received an ip injection of 0.25 nmol/g bw of a mutant midsegment. Compared with vehicle controls, mice that received M30 MSH-A had a significantly reduced food intake (0.97 ± 0.11 *vs.* 0.68 ± 0.07 g) (Fig. 8B). This suggests that the midsegment region homologous to that of α-MSH is not required

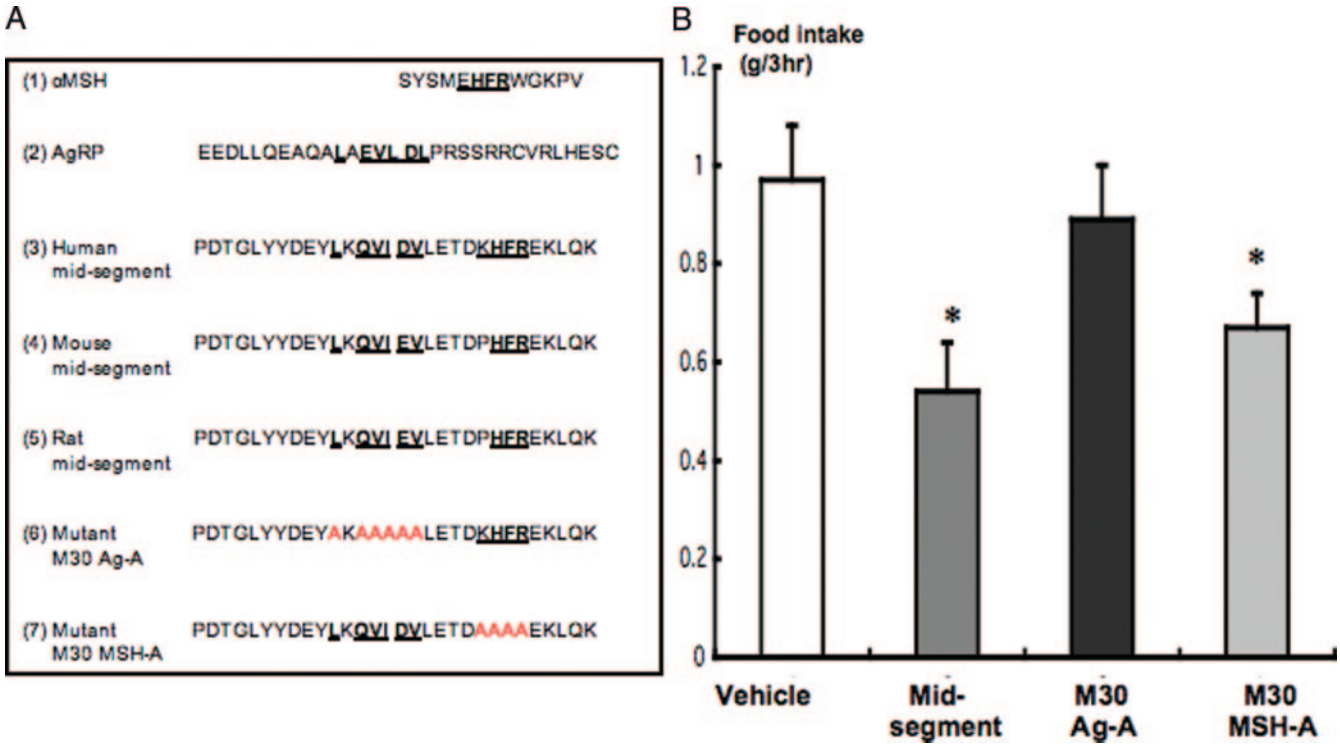

FIG. 8. Effect of mutant midsegments on food intake. A, Alignment of the amino acid sequence of the midsegment with those of α-MSH and AgRP identified regions of similarity (*underlined*); the amino acids replaced by alanine residues are shown in *red* in mutant M30. B, Mice were given a 0.25 nmol/g bw ip injection of each mutant midsegment, and food intake for 3 h was determined. Each group consisted of seven to 14 mice. Data are expressed as means ± SEM. *, $P < 0.01$ *vs.* vehicle administration.

for anorexigenic induction. In contrast, no significant reduction in food intake was seen in mice that received M30 Ag-A.

Discussion

We previously demonstrated that nesfatin is expressed in the feeding centers of the rodent brain (13). Neurons in the paraventricular nucleus of the hypothalamus that express nesfatin are inactivated by starvation and activated after refeeding (13, 15). Nesfatin is processed to an active derivative, nesfatin-1, that is present in the cerebrospinal fluid of rats. In a preliminary study using a sandwich-type ELISA, we confirmed that nesfatin-1 was present in the blood of rodents and human (data not shown here). Although the origin of circulating nesfatin-1 in the general circulation is still unknown, circulating nesfatin-1 may cross the blood-brain barrier (16, 17) and be involved in the central regulation of feeding behavior. In this study, we examined whether peripherally administered nesfatin-1 affected food intake in mice.

Peripheral injection of nesfatin-1 caused significant decreases in food intake in mice but did not cause any toxic or aversive effects. Of the three segments derived from nesfatin-1, only the midsegment proved effective. The IC_{50} for reducing feeding was indistinguishable between nesfatin-1 and the midsegment and was nearly equivalent to values for an α-MSH analog and leptin. The amino acid sequence of the midsegment is well conserved in different species, and peptides corresponding to the mouse, rat, or human midsegments showed equal potency. These results indicate that the middle region of nesfatin-1 contains a site essential for anorexigenic function. Because the midsegment is predicted to be α-helix rich, our data appear compatible, at least in part, with previous observations that an α-helical structure in endogenous ligands is essential for interaction with the relevant receptors (37, 38).

Previously, we identified the leptin-independent signaling system that contributes to feeding suppression induced by central nesfatin-1 (13, 39). In a similar fashion, the present data demonstrated that peripheral injection of the midsegment was effective under leptin-resistant conditions, such as in db/db mice and mice fed a high-fat diet, indicating that peripheral signaling of nesfatin-1 also exhibits leptin independence. We next examined whether peripheral signaling by nesfatin-1 takes place at brain regions that differ from those observed for leptin signaling. Peripheral signaling by leptin is mainly initiated by activation of anorexigenic POMC and CART neurons in the hypothalamic Arc via a humoral route. In contrast, POMC and CART neurons in the brainstem NTS are not activated by peripheral leptin (5, 40). The present data demonstrate that ip injection of the nesfatin-1 midsegment activated c-Fos-positive neurons in the NTS and area postrema but not in the Arc. Moreover, it is clear from the present data that the nicotinic cholinergic pathway to neurons in the NTS involved the peripheral induction of anorexia by the midsegment. The NTS and area postrema receive and integrate peripheral vagal anorexigenic signals and relay the signal to hypothalamic feeding centers, such as the paraventricular and supraoptic nuclei (41, 42). Our observations here indicate that peripherally administered midsegment affects the NTS and area

postrema directly or indirectly. After injection of the midsegment, the NTS area showed significant increases in CART and POMC gene expression, whereas expression of these genes was not activated in the Arc. Taken together with observations that POMC overexpression of neurons in the NTS, in addition to the Arc, results in hypophagia (43, 44), the present observations imply that peripheral signaling by nesfatin-1 activates neurons expressing anorexigenic molecules POMC and CART in the NTS to produce anorexia, in a manner dissimilar to leptin signaling. It will be necessary to perform experiments using a selective blocker of melanocortin signaling to obtain conclusive evidence that the NTS melanocortin pathway mediates the effect of the nesfatin-1 midsegment. We found that the region of the midsegment that shows similarity to α-MSH was not responsible for inducing anorexia. Contrary to expectations, the region of the midsegment with similarity to AgRP was essential for anorexigenic induction. AgRP is an endogenous antagonist for MC4R (45), and previous findings have shown that it has diverse sequences responsible for increased or decreased energy homeostasis (36, 46). The midsegment contains a region with sequence similarity to a sequence in AgRP exhibiting increased energy homeostasis.

Based on the present studies, it should be feasible to develop a number of nesfatin-1 analogs that have an anorexigenic function.

Acknowledgments

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