Mini-Review

The Role of Central Neurotensin in Regulating Feeding and Body Weight

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Abbreviations: GABA, γ-aminobutyric acid; GLP-1, glucagon-like peptide 1; GPCR, G-protein–coupled receptor; ICV, intracerebroventricular; LHA, lateral hypothalamic area; NAc, nucleus accumbens; NMN, Neuromedin; Nts, neurotensin; NtsR, neurotensin receptor; SN, substantia nigra; VTA, ventral tegmental area.

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Abstract

The small peptide neurotensin (Nts) is implicated in myriad processes including analgesia, thermoregulation, reward, arousal, blood pressure, and modulation of feeding and body weight. Alterations in Nts have recently been described in individuals with obesity or eating disorders, suggesting that disrupted Nts signaling may contribute to body weight disturbance. Curiously, Nts mediates seemingly opposing regulation of body weight via different tissues. Peripherally acting Nts promotes fat absorption and weight gain, whereas central Nts signaling suppresses feeding and weight gain. Thus, because Nts is pleiotropic, a location-based approach must be used to understand its contributions to disordered body weight and whether the Nts system might be leveraged to improve metabolic health. Here we review the role of Nts signaling in the brain to understand the sites, receptors, and mechanisms by which Nts can promote behaviors that modify body weight and whether the Nts system might be leveraged to improve metabolic health. Here we review the role of Nts signaling in the brain to understand the sites, receptors, and mechanisms by which Nts can promote behaviors that modify body weight and whether the Nts system might be leveraged to improve metabolic health. Defining the central mechanisms by which Nts signaling modifies body weight may suggest strategies to correct disrupted energy balance, as needed to address overweight, obesity, and eating disorders.

Key Words: feeding, physical activity, lateral hypothalamic area, neurotensin receptor, obesity, anorexia

At just 13 amino acids in length, neurotensin (Nts) is a small peptide that exerts a large impact on physiology. Nts signaling is implicated in pain perception, reward processing, psychiatric disease, sleep, muscle contraction, blood pressure, thermoregulation, and, more recently, the regulation of body weight (1-3). This physiological laundry list begs the question: How can a single peptide mediate such a diverse array of physiology and behavior?
Thanks to new methods that identify and modulate specific cells of the Nts system an answer is emerging: Function varies with the location of Nts signaling. Recent reports illuminate site-specific Nts mechanisms that mediate specific aspects of physiology (4-9), and the race is on to match Nts- and Nts receptor–expressing cells throughout the body with their precise functions. This parceled Nts function also provides insight into control of body weight, namely why Nts in the periphery can support weight gain but central Nts signaling favors weight loss. For Nts (as for real estate) location matters, and thus location must be carefully considered to weigh the effects on energy balance.

**Role of Neurotensin in the Periphery on Body Weight**

Although Nts was originally isolated from the bovine brain, early work focused on its peripheral actions, where it can be hypotensive (10) and induce smooth-muscle contraction including gut motility (11). Most peripheral Nts is produced by the adrenal gland (12) and a subset of enteroendocrine cells (13), and these sources provide the large pool of circulating Nts that can access multiple tissues. The short half-life of Nts in circulation, only 30 seconds in rodents, has made it difficult to study its role as an endocrine signal (14). However, ingesting dietary fat causes a rapid, transient elevation of Nts in the plasma (15, 16), likely released from enteroendocrine cells (17). Nts promotes intraluminal fat absorption, which may explain why whole-body Nts knockout mice have reduced lipid uptake and are protected from developing diet-induced obesity (9, 18). The peripheral Nts system seems directly coordinated with energy status, so that plasma Nts rapidly increases after ingestion (19, 20), usually returning to basal levels hours later.

Given that Nts coordinates dietary fat intake and intestinal lipid absorption, dietary changes can affect peripheral Nts function and body weight. Loss-of-function mutations in Nts have been described in patients with anorexia nervosa, a disorder characterized by self-restricted feeding and dangerously low body weight (21). Reduced peripheral Nts may conceivably limit fat absorption that, along with self-imposed caloric restriction, reduces body weight. Alternatively, peripheral Nts may interact with modern, fat-rich diets to potentiate fat absorption, weight gain, and associated metabolic sequelae. Elevated plasma Nts has been correlated with the risk of developing obesity, type 2 diabetes, and obesity-related liver and cardiovascular disease, supporting the interpretation that Nts promotes weight gain (Table 1). Counterintuitively, obese individuals who have undergone bariatric surgical procedures and weight loss exhibit further elevations in circulating and intestinal levels of Nts (Table 2). If plasma Nts levels predict potential for fat uptake and weight gain, why would bariatric procedures and ensuing weight loss result in increased Nts levels? One possibility is that Nts production is elevated to compensate for lipid malabsorption induced by bariatric procedures. Since recommended postprocedure diets are low in fat, elevated plasma Nts may not be sufficient to enhance fat accumulation. Meanwhile, a side effect of increased plasma Nts is increased transport across the blood-brain barrier, augmenting Nts levels in hypothalamic regions that regulate energy balance (22-24). Whereas Nts in the intestine favors fat absorption and weight accumulation, Nts acts within the brain to reduce feeding and increase physical activity behaviors that favor weight loss. Hence, it is possible that extremely elevated plasma Nts levels after bariatric procedures permit enough Nts access to the brain to invoke centrally mediated behaviors that support weight loss (24). Defining the physiological role of circulating Nts after bariatric procedures may advance understanding of how and where these surgeries promote weight reduction. An outstanding question is whether plasma Nts is a specific biomarker for metabolic disease or a generalized disease marker. Elevated plasma Nts has also been noted in nonobese individuals with breast and colon cancer, suggesting that Nts levels may not solely reflect adiposity or proclivity for weight gain (25, 26). While there are clearly important Nts mechanisms governing body weight regulation via the periphery, the rest of this review focuses on the role of the central Nts system on energy balance, and its potential to address weight disorders.

**Central Neurotensin Modulates Behaviors That Affect Body Weight**

 Elevated plasma Nts can access some blood-brain barrier–adjacent brain regions that contribute to satiety, but not deeper brain sites enriched in Nts receptors and implicated in suppressing feeding. Instead, the majority of centrally acting Nts is produced within the brain and acts as a neuropeptide transmitter. Accumulating evidence supports that some, but not all, central Nts neurons and their neurotensin receptor-expressing targets restrain feeding while supporting physical activity and energy expenditure. Defining the specific Nts neurons, receptors, and circuits that modulate these behaviors may inform new strategies to treat metabolic disease and eating disorders. Here we review the literature on Nts in central regulation of feeding and physical activity, and how disruption of Nts action may contribute to altered energy balance and body weight.
What Is the Pharmacological Effect of Neurotensin on Feeding and Body Weight?

Large doses of peripherally administered Nts and the structurally related peptide xenin transiently attenuate feeding in fasted, hungry rodents (22, 27). The brevity of the anorectic effect may be attributed to the short half-life of Nts and xenin peptides in circulation. Comparatively, systemic treatment with a more stable form of Nts produced robust, long-lasting reduction of feeding (22, 28). Intriguingly, similar reductions in feeding are observed after administering low-dose Nts or xenin into the brain (via intracerebroventricular [ICV] injection), indicating that the brain is sufficient to mediate the anorexigenic actions (27-29). Nts and xenin have more potent anorectic effects in rodents that are diet-induced or genetically obese, which might be useful to support weight reduction (30, 31). However, ICV Nts or xenin treatment also attenuates locomotor activity and promotes resting behavior (28, 32, 33), countering the anorectic effect and dampening possible weight loss. These hypolocomotor effects are likely exerted by direct actions of Nts in the nucleus accumbens (NAc), where Nts has been shown to diminish psychostimulant-mediated locomotor activity (34, 35). Additionally, ICV Nts causes other effects that are not necessary for modulating body weight, and dangerously decreases blood pressure and body temperature (36-38). Thus, brain-wide Nts administration exerts an anorectic effect that might be useful to support weight loss, but also clinical liabilities that must be avoided.

Pharmacological administration of Nts into specific brain regions suggests that there are Nts circuits to modulate discrete functions, only some of which are dedicated to feeding and activity behaviors that affect body weight. For example, Nts injection within the periaqueductal gray invokes analgesia but has not been reported to alter feeding (1, 2). In contrast, Nts injections into the ventral tegmental area (VTA) (58-60) or substantia nigra (SN) (61) decrease feeding. Nts also exerts site-specific effects on locomotor activity: Nts administration into the VTA (62, 63) and hippocampus (64) increases locomotion, whereas Nts in the NAc decreases general locomotor activity (34, 65) and psychostimulant response (65, 66) without any influence on feeding (28). Notably, the VTA, SN, NAc, and hippocampus are all part of the limbic system, where dopamine

| Disease                                         | Subject                      | Plasma Nts/xenin concentration (compared to control) | Method of detection | References |
|------------------------------------------------|------------------------------|-----------------------------------------------------|--------------------|------------|
| All-cause mortality                            | Human                        | Higher pro-Nts                                      | ELISA              | (39, 40)   |
| Obesity                                        | Human                        | No change Nts                                       | ELISA              | (41)       |
| Obesity                                        | Human children               | Lower xenin                                         | RIA                | (42)       |
| Obesity                                        | Human                        | Higher pro-Nts                                      | ELISA              | (9)        |
| Obesity                                        | Human                        | Higher xenin                                        | RIA                | (43)       |
| Visceral adipose tissue inflammation           | Human                        | Higher pro-Nts                                      | ELISA              | (44)       |
| Diabetes—type 2                                | Human                        | Lower pro-Nts                                       | ELISA              | (45)       |
| Diabetes—gestational                           | Human                        | No change pro-Nts                                   | ELISA              | (46)       |
| Diabetes                                       | Human females                | Higher pro-Nts                                      | ELISA              | (16)       |
| Diabetes                                       | Human                        | Higher pro-Nts                                      | ELISA              | (44, 47)   |
| Cardiovascular disease                         | Human                        | Higher pro-Nts                                      | ELISA              | (16, 39)   |
| Coronary heart disease                         | Human                        | Higher pro-Nts                                      | ELISA              | (47)       |
| Cardiovascular disease: mortality              | Human                        | Higher pro-Nts                                      | ELISA              | (39, 40, 47)|
| Nonalcoholic fatty liver disease and obesity   | Human                        | Higher pro-Nts                                      | ELISA              | (15)       |
| Nonalcoholic fatty liver disease and obesity   | Human                        | Lower Nts                                            | ELISA              | (41)       |
| Hyperthyroidism                                | Human                        | Higher Nts                                           | RIA                | (48)       |
| Hypothyroidism                                 | Human                        | Lower Nts                                            | ELISA              | (48)       |
| Irritable bowel disease                        | Human children               | No change in xenin                                   | RIA                | (42)       |
| Prader-Willi syndrome                          | Human                        | Higher Nts                                           | ELISA              | (49)       |
| Anorexia                                       | Human                        | More Nts degradation                                 | Fluorimetry of enzymatic activity | (50) |

Summary of literature on the amount of plasma Nts or xenin during disease states linked with altered body weight. Abbreviations: ELISA, enzyme-linked immunosorbent assay; Nts, neurotensin; pro-Nts, proneurotensin/neuromedin precursor peptide that is more stable in circulation; RIA, radioimmunoassay.

Table 1. Circulating neurotensin and xenin levels in metabolic disease and anorexia
modifies the motivational, goal-directed pursuit of drug and natural (food) rewards (67, 68). Hence, these data support the view that at least some Nts-mediated anorectic effects occur because of engaging and altering dopamine signaling. Nts also acts in specific hypothalamic regions involved in energy homeostasis. For example, the subset of neurons in the hypothalamic arcuate nucleus that coexpress Nts and neuropeptide Y influence body weight, although the role of Nts via these neurons is yet unknown (69). Nts also directly activates arcuate nucleus neurons that are part of the melanocortin system, and at least some of the elicited anorectic effect depends on melanocortin signaling (22, 24). Nts delivery to other homeostatic centers, including the ventromedial hypothalamus (70), the paraventricular hypothalamic nucleus (71), or the nucleus tractus solitarius (72) also decreases food intake. Hence, Nts can specifically modulate feeding and promote weight loss via dopaminergic and homeostatic (nondopaminergic) brain regions. Given that pharmacological Nts administration in these discrete brain regions favors weight loss behaviors, there may be dedicated subsets of Nts neurons and circuits that affect energy balance without disturbing other physiology.

How Does Endogenous Central Neurotensin Regulate Body Weight?

Genetically or diet-induced obese rodents have reductions in the central processing and expression of Nts (73-76) suggesting that loss of Nts signaling might contribute to the development of overeating. Yet, the site-specific pharmacological effects of Nts suggest that only certain Nts neurons modulate Nts expression, and/or Nts-mediated feeding, movement, and body weight. Parsing endogenous Nts-expressing neurons was long thwarted by technical challenges in detecting Nts. Typically, peptide-expressing neurons can be visualized via antibody-mediated immunoreactivity, but this technique identifies Nts only within axons and terminals. To detect Nts-expressing soma rodents must be pretreated with ICV colchicine, an axonal transport inhibitor that causes sufficient Nts accumulation within soma to enable immunodetection. Colchicine enables visualization of Nts-expressing soma but impairs neuronal function and leads to death within 48 hours, so it precludes investigation of normal Nts-mediated physiology and behavior. The development of mice that express cre-recombinase in Nts neurons (Nts Cre mice) has simplified visualization and modulation of endogenous Nts-expressing neurons. Nts Cre mice, similar to in situ hybridization, reveal dense subpopulations of Nts neurons throughout the brain, such as within the lateral hypothalamic area (LHA), the lateral septum, the medial preoptic area, the dorsal subiculum, and

| Table 2. Effect of gastric bypass and banding on neurotensin and xenin levels |
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| **Surgery** | **Reference** |
| RYGB Humans 25-53 mo | Decrease Higher pro-Nts/NMN Weight-matched individuals Plasma ELISA (51) |
| RYGB Humans 3, 6, 12, and 24 mo | Decrease Higher pro-Nts/NMN Presurgery Plasma ELISA (52) |
| RYGB Rats 10-11 mo | Decrease Increase of Nts-expressing cells Weight-matched individuals Gut IHC (53) |
| RYGB Rats 23 wk | Decrease Higher Nts Weight-matched individuals Plasma RIA (22) |
| RYGB Humans 1 y | Decrease Higher pro-Nts Presurgery Plasma ELISA (54) |
| RYGB Humans 8 mo | Decrease Higher xenin Presurgery Plasma RIA (43) |
| RYGB Humans 8 mo | Decrease Higher xenin Presurgery CSF RIA (43) |
| Biliopancreatic diversion with duodenal switch Humans 1 y | Decrease Higher pro-Nts Presurgery Plasma ELISA (54) |
| JIB Humans 9 mo | Decrease Higher pro-Nts Presurgery Plasma RIA (55) |
| JIB Humans 20 y | Decrease Higher pro-Nts Weight-matched individuals Plasma RIA (56) |
| GB Humans 203-355 d | Decrease Higher Nts Presurgery Plasma RIA (57) |

Summary of literature on the impact of gastric surgical procedures on Nts and xenin. Abbreviations: ELISA, enzyme-linked immunosorbent assay; GB, adjustable gastric banding; JIB, jejunoileal bypass; NMN, Neuromedin; pro-Nts, proneurotensin/neuromedin precursor peptide that is more stable in circulation; RIA, radioimmunoassay; RYGB, Roux-en-Y gastric bypass.
central amygdala (77). Work from NtsCre mice has begun to match these site-specific Nts neurons to control of social behavior (medial preoptic area), memory (dorsal subiculum), and ethanol consumption (central amygdala) (5, 6, 78). In most of these studies Nts was used as a genetic marker to modulate a neuronal population but the physiological role of Nts was untested. By contrast, Nts-expressing neurons of the LHA suppress feeding and support weight loss via an Nts receptor-dependent mechanism (4). Lateral septum Nts neurons also project to and mediate anorectic effects via the LHA. Hence, the LHA is a common “hub” for Nts-mediated feeding suppression (4, 79). Curiously, whole-body Nts knockout mice exhibit reduced intestinal fat absorption but not alteration in feeding or physical activity, as might be expected because of the loss of Nts in the brain (9). These data suggest that central Nts is not required for control of feeding, though it is sufficient to modify it. The distribution of Nts neurons supports this view, as they are primarily found in limbic-related brain regions that modulate feeding rather than in hypothalamic nuclei critical for homeostasis (eg, the arcuate, ventromedial hypothalamus, dorsomedial hypothalamus).

The anorectic role of Nts-expressing LHA neurons is intriguing because these neurons release Nts to the VTA (80-82), a site where Nts diminishes feeding and promotes physical activity. Moreover, activation of LHA neurons is sufficient to modulate feeding and movement (83). LHA Nts neurons are a specific subset of all LHA neurons that project widely throughout the brain and release both Nts and γ-aminobutyric acid (GABA) (4, 80). Some LHA Nts neurons also express the long form of the leptin receptor and are vital for leptin-mediated regulation of body weight (81). Lesion of LHA Nts neurons promotes adiposity, whereas activation of LHA Nts neurons suppresses feeding and promotes arousal, physical activity, and energy expenditure that yield weight loss (4, 84-86). These effects occur in part via Nts-mediated regulation of VTA dopamine neurons (4, 80), but other projection sites may also contribute to Nts-regulated feeding. Notably, activation of LHA Nts neurons does not cause hyperthermia associated with other Nts systems, and in fact invokes mild hyperthermia likely due to elevated physical activity (8, 85). It has yet to be determined whether LHA Nts neurons modulate blood pressure or pain processing, but they promote voracious drinking independent of Nts signaling (4). Given that this burst of drinking acutely increases weight, it may initially deter the weight loss effect of LHA Nts neurons. There are suggestions that LHA Nts-mediated suppression of feeding and promotion of drinking are regulated by different subsets of LHA Nts neurons and circuits (86). If true, it may be possible to selectively augment the “anorectic circuit” without invoking the “drinking circuit,” which could potentiate weight loss. It is equally possible that some LHA Nts neurons coordinate drinking and feeding suppression, such as dehydrated animals prioritizing water-seeking above feeding, termed dehydration anorexia. The increased Nts expression and neural activity observed within the LHA during dehydration anorexia suggests that LHA Nts neurons play an active role in coordinating osmotic status and ingestive behavior (86, 87). It also remains to be determined how other transmitters expressed within LHA Nts neurons contribute to their function, including anorectic corticotropin-releasing hormone, or galanin and GABA implicated in promoting feeding via the LHA and VTA (88-91). Some LHA Nts neurons have also been reported to contain glutamate, but this appears to be a separate, more rostrally located population compared to the large population of Nts-GABA neurons found in the perifornical LHA (80-82). Thus, at least some dedicated, endogenous Nts populations exist that may mediate anorexia but not other physiology.

Leptin Engages Central Neurotensin Signaling to Modulate Body Weight

The brain receives multiple energy cues from the circulation that convey energy status, including levels of glucose and hormones of energy sufficiency (leptin, glucagon-like peptide 1 [GLP-1]), and energy deficit (ghrelin) (84, 87, 92). While ghrelin, insulin, or glucose do not directly act on Nts-expressing neurons, there is evidence that the anorectic hormone leptin modulates the central Nts system. Leptin increases Nts expression (93) but rodents that genetically lack leptin or its receptors have diminished central Nts expression along with obesity (73-76). More recently, the site of leptin and Nts interaction was pinpointed to the LHA, where a subset of Nts neurons coexpress the long form of the leptin receptor and are activated by leptin (81, 92, 94). Loss of either leptin receptor or Nts signaling via these LHA neurons attenuates leptin-mediated suppression of feeding and promotes obesity (81, 84, 92, 95). Leptin specifically activates LHA Nts neurons that project to and modulate the activity of orexin and dopamine neurons to alter feeding and physical activity (95-97). Currently, the LHA Nts neurons appear to be the unique location via which leptin and Nts action intersect. Other Nts-expressing neurons that regulate feeding (eg, lateral septum, arcuate nucleus) do not express leptin receptor, and so are not direct mediators of leptin action (79, 81). There is also some interaction of Nts and GLP-1 since coadministration of these peptides synergizes weight loss beyond treatment with either peptide alone (24). Going forward, it will be important to discern the endogenous cues that modulate Nts neurons to understand how they contribute to feeding, movement, and body weight.
Central Neurotensin Receptors and Weight Modulation

Nts acts via binding to 3 receptors: neurotensin receptor-1 (NtsR1), -2 (NtsR2), and -3 (NtsR3/sortilin) (98). NtsR1 and NtsR2 are G-protein–coupled receptors (GPCRs), via which Nts binding alters G\textsubscript{q} and β-arrestin coupling to change intracellular signaling and neuronal activity. NtsR3/sortilin is not a GPCR, but rather a single-pass transmembrane neuro peptide receptor that plays a role in intracellular sorting of proteins (99). All 3 receptor isoforms are found within the brain but differ in Nts binding affinity, structure, and distribution, which suggests that they contribute to different aspects of Nts-mediated physiology (1). Although NtsR3/sortilin may contribute to Nts-mediated analgesia and other physiology involving (100, 101) and excluding (102) Nts signaling, it has not yet been linked with control of body weight. There are also Nts receptors in the periphery that play a role in gut motility and plausibly energy balance (103, 104), but here we focus on the roles for central NtsR1 and NtsR2 in feeding and physical activity behaviors that can affect energy balance.

Neurotensin Receptor-1

NtsR1 is the high-affinity receptor for Nts and also binds the structurally related peptide xenin (105). NtsR1 is expressed on the plasma membrane of a subset of adult brain neurons (106). It is the only Nts receptor isoform that has been explicitly linked to energy balance and body weight, but NtsR1 also mediates many other functions of neonatal development (107), analgesia (108), reward, and cardiorespiration (1). Structural analysis of NtsR1 suggests that this GPCR may couple to G\textsubscript{q/11} (109-111), G\textsubscript{i/0} (109, 110, 112), G\textsubscript{i/q} (109, 110, 112), and β-arrestins (113). Consistent with general GPCR signaling, the coupling of NtsR1 determines the intracellular effect of Nts binding. G\textsubscript{i/o} coupling increases inositol phosphatase activity (114-116) that increases intracellular calcium and cell-firing (117), as well as activation of protein kinase C (118, 119). By contrast, G\textsubscript{q/11} coupling can reduce cAMP (3′,5′-cyclic adenosine 5′-monophosphate), whereas β-arrestins will guide receptor internalization (109, 120-123). Importantly, all of these coupled pathways can influence neuronal activity and function, but with very different consequences for cells. It is possible that NtsR1-expressing neurons in different brain regions may couple to different pathways, which might explain the varied reports of how NtsR1 alters cell signaling and function. This hypothesis, however, has been difficult to explore because of the lack of reagents to identify NtsR1 neurons to study their specific Nts-mediated signaling pathways. Recently established genetic models now permit site-specific modulation of NtsR1-expressing cells that may assist the field in defining the coupling of NtsR1 across different brain regions (1, 98, 124, 125), and thus the mechanism by which Nts-NtsR1 signaling modulates physiology.

NtsR1 is generally accepted as the Nts receptor isoform that modulates feeding. Indeed, pharmacologic Nts treatment does not suppress feeding in NtsR1-deficient mice, confirming the necessity of NtsR1 for Nts-mediated anorectic response (4, 126, 127). Yet, use of the constitutive NtsR1-knockout line has yielded equivocal interpretations of how NtsR1 contributes to energy balance, either promoting or not changing feeding and body weight (97, 126, 127). Such studies should be interpreted with caution because developmental deletion of NtsR1 may cause organizational changes and compensation that do not reflect the normal physiology of NtsR1 in the adult brain. It is now recognized that NtsR1 is widely but transiently expressed throughout the brain during early stages of development (128, 129), yet NtsR1 is retained by only a few select neuronal populations in the adult brain (77). These data hint at a developmental role for NtsR1, perhaps in establishing neuronal circuitry and systems, hence constitutive NtsR1-knockout mice likely have extensive disruptions in neural circuitry. Pharmacological data also support a role for NtsR1 in regulating behaviors that affect body weight. NtsR1 agonists suppress feeding (31) but NtsR1 antagonists block the anorectic and locomotor effects of Nts in adult rodents (4, 80, 97). Hence, pharmacologic modulation of NtsR1 could be used to modify feeding and possibly body weight. However, systemic or central Nts or NtsR1 agonists also produce dangerous hypothermia and vasodepression (31). It remains to be determined whether there are dissociable, NtsR1-expressing populations that mediate these effects vs feeding.

The limited sites of NtsR1 expression in the adult brain suggest that this receptor contributes to specific aspects of Nts action, and via dedicated circuits. For example, NtsR1 is expressed within the olfactory tubercle, the arcuate nucleus and the bed nucleus of the stria terminalis (1, 98, 130), though it is unclear how Nts-NtsR1 signaling via these regions modifies feeding or energy balance. NtsR1 is also highly expressed by some dopamine neurons of the VTA and SN, where it is coupled to G\textsubscript{i/o} (109, 117). Hence, Nts can directly activate NtsR1-expressing dopamine neurons, which increases their activation (117, 131) and promotes dopamine release to the NAc (132, 133), where dopamine modulates feeding, movement, and body weight. Indeed, the interaction of NtsR1 and dopamine is critical, as either genetic ablation of VTA NtsR1 neurons or treatment with NtsR1 antagonists alters dopamine signaling, locomotor activity, response to feeding hormones, and...
body weight (80, 134). NtsR1 deficiency also causes alterations in dopaminergic signaling and blunts the ability of LHA Nts neurons to suppress feeding and promote locomotor activity (4). Since LHA Nts neurons project to and modulate VTA dopamine neurons, these data support an LHA Nts→VTA NtsR1:dopamine circuit in the regulation of body weight. Curiously, other Nts-expressing neurons provide input to the VTA and modify social or locomotor behavior but not feeding (5). It remains unknown how various sources of Nts input elicit different effects in the VTA, but it could be due to engaging distinct neurons and/or different Nts receptor isoforms. Moreover, it remains to be determined whether all VTA NtsR1 neurons specifically mediate weight loss, or whether they also mediate Nts-stimulated analgesia, hypothermia, and vasodepressor response. Future studies will be crucial for evaluating the potential of modulating NtsR1 signaling for treating disordered body weight.

Neurotensin Receptor-2

NtsR2 has lower affinity for Nts compared to NtsR1 but is more widely expressed in the brain (1, 98, 124, 125). NtsR2 is expressed within regions that contribute to feeding, movement, and body weight, such as the hippocampus, hypothalamus, bed nucleus of the stria terminals, and the VTA (1, 98, 124, 125). However, NtsR2 is predominantly expressed in astrocytes rather than neurons (135-138) though its role via astrocytes is unclear. Astrocytes have recently been recognized as important modulators of feeding, metabolism, and body weight (139), raising the possibility that NtsR2-expressing astrocytes may contribute to these processes. Indeed, in the preoptic area Nts invokes hypothermia via the collective actions of NtsR2-expressing astrocytes and adjacent NtsR1-expressing neurons that signal via different mechanisms (135).

A role for NtsR2 in energy balance was originally dismissed because mice constitutively lacking NtsR2 have normal feeding and body weight (98). NtsR2-deficient mice also suggested a facilitatory role for NtsR2 in thermal nociception (140), but pharmacological studies support an analgesic role of NtsR2 (141-143). Given these discrepancies, along with concerns that constitutive knockout mice do not reflect normal physiology, a role for NtsR2 in controlling body weight is not necessarily excluded. Indeed, the abundance of NtsR2 within the VTA and SN (1, 98, 124, 125), where Nts treatment is known to suppress feeding and regulate locomotor activity, suggests that Nts may exert some part of these behaviors via NtsR2 as well as NtsR1. It has also been speculated that differences in Nts concentration might bias signaling via Nts receptors to produce opposite physiological responses. For example, low concentrations of Nts increase excitatory signaling in the VTA via NtsR1, whereas high Nts concentration reduces excitatory signaling via an NtsR2-dependent mechanism (82). Going forward, use of genetic tools to parse the cell-type specific roles of NtsR2 astrocytes vs NtsR1 neurons will be important to reveal their functions.

Translational Potential of the Central Neurotensin System for Modulating Body Weight

Plasma Nts is increased in individuals with obesity and Prader-Willi syndrome, suggesting that disrupted Nts signaling may contribute to and perhaps serve as a diagnostic marker for these disease states (see Table 1). Given that Nts function differs in the periphery and brain, it is necessary to separately evaluate the role of central Nts in disordered body weight. In contrast to the elevated plasma Nts observed in obesity, most experimental models of obesity exhibit decreased expression of Nts in the brain (Table 3). Moreover, pharmacological and in vivo enhancement of Nts signaling beneficially modifies feeding, moving, and body weight in experimental models. This begs the question: Can the central Nts system be clinically leveraged to treat disordered body weight? Currently there are no approved drugs to safely modify the Nts system without invoking dangerous hypothermia and vasodepression. Brain-penetrant Nts analogues and NtsR1- or NtsR2-targeted drugs have been generated, but have only been tested experimentally. NtsR2 agonists are being examined for potential to treat chronic pain since they do not disrupt body temperature or blood pressure, but neither do they appear to regulate feeding or body weight (144). While NtsR2 is not directly linked with disordered body weight, it might be leveraged in treating a common complication of obesity: diabetic peripheral neuropathy (145). Indeed, Nts or activation of NtsR1 or NtsR2 alleviates neuropathic pain (146-148). Thus, NtsR2-biased agonists might be useful to provide analgesia in individuals with obesity and diabetic neuropathy, although they may not modify metabolic aspects of the disease. Systemic treatment with brain-penetrant Nts analogues and NtsR1 agonists promotes weight loss in rodents and primates, but also hypothermia and vasodepression (31, 149, 150). These serious clinical liabilities diminished enthusiasm in modulating the Nts or NtsR1 system. Combinatorial treatments of Nts and a GLP-1 mimetic (liraglutide), or of xenin and various gut peptides have recently been explored in preclinical obesity models. These combinatorial approaches show promise in modifying body weight and glycemic response, although it is unclear whether they also promote adverse physiologic liabilities (24, 151).
However, use of genetic tools to modulate specific Nts circuitry now suggests that activating certain subsets of Nts or NtsR1-expressing neurons mediates specific physiology without invoking adverse effects (4-8, 79). Of these, activation of LHA Nts neurons that release Nts to the VTA promotes beneficial weight loss behaviors, but not adverse physiological effects or reinforcement (4-8, 85). It is possible that using new methods to modulate NtsR1- and NtsR2-expressing cells may reveal region- and cell-specific populations that modify feeding and body weight without clinical liabilities. Systematically defining the key brain locations, receptors, and signaling mechanisms by which Nts modulates energy balance, along with brain-permeable agonists for NtsR1 or NtsR2 (144, 155, 156) could permit tailoring drugs to selectively enhance Nts action in specific sites. For example, there are preclinical compounds selective for NtsR1-dopamine-2-receptor heterodimers found in the VTA (157). Although it is unclear how NtsR1-D2R heterodimers signal, they might be used to target compound delivery to VTA NtsR1 neurons, where Nts suppresses feeding and supports physical activity. Better understanding of the signaling pathways invoked by NtsR1 may also suggest clinical opportunities to safely potentiate NtsR1-mediated anorectic effects. For example, recently developed NtsR1 agonists biased for the β-arrestin-2 pathway do not produce cardiovascular liabilities (156) but reduce rodents’ intake of psychostimulants in a dopamine-dependent manner (156, 158). While β-arrestin-2-biased agonists for NtsR1 have yet to be studied in regulating natural reward intake, these data reinvigorate the possibility of modulating beneficial Nts-NtsR1 signaling without endangering health.

The recent discovery of loss-of-function mutations in Nts and NtsR1 in patients with anorexia nervosa hints at their contribution to eating disorders (21). The risk for developing eating disorders is complex, due to a combination of developmental timing, social, sex, stress, biological, and genetic factors. Given the anorectic role of the Nts system in adult brains, it seems counterintuitive that loss of Nts-NtsR1 signaling would reduce individuals’ feeding behavior. However, NtsR1 may exert different roles in the adult vs developing brain, particularly within the dopamine system that is known to modulate feeding. NtsR1 is expressed by a subset of adult dopamine neurons whereby Nts-NtsR1 signaling in the established brain restricts feeding. The dopamine system is heightened in anorexia nervosa, and is thought to contribute to the feeding restriction and excessive exercise behaviors characteristic of the disorder (21, 50), but the cause of heightened dopamine circuitry remains unknown. Intriguingly, NtsR1 is implicated in ontogeny of the brain, is transiently expressed by all developing midbrain dopamine neurons, and

### Table 3. Central neurotensin and xenin expression in metabolic disease and anorexia

| Disease                  | Subject  | Central Nts or xenin | Location               | Method of detection | References |
|--------------------------|----------|----------------------|------------------------|---------------------|------------|
| Obesity                  | Human    | Higher xenin         | CSF                    | RIA                 | (43)       |
| Obesity                  | Rat      | Lower Nts            | Dorsomedial nucleus    | RIA                 | (152)      |
| Obesity                  | Rat      | Lower Nts            | Arcuate nucleus        | RIA                 | (152)      |
| Obesity                  | Rat      | Lower Nts            | Median eminence        | RIA                 | (152)      |
| Obesity                  | Rat      | Increased Nts        | LHA                    | RIA                 | (152)      |
| Obesity                  | Rat      | Lower Nts            | Ventromedial nucleus   | RIA                 | (75)       |
| Obesity                  | Rat      | Lower Nts            | Arcuate nucleus        | RIA                 | (75)       |
| Obesity                  | Rat      | Lower Nts            | Median eminence        | RIA                 | (75)       |
| Obesity                  | Rat      | Lower Nts            | Suprachiasmatic nucleus| RIA                 | (75)       |
| Obesity                  | Rat      | Lower Nts            | Paraventricular nucleus| RIA                 | (75)       |
| Obesity                  | Mouse    | Lower Nts            | LHA                    | RIA                 | (73)       |
| Obesity                  | Mouse    | Lower Nts mRNA       | Central hypothalamus   | RIA                 | (73)       |
| Hyperthyroidism          | Rat      | No change in Nts mRNA| Lateral hypothalamus   | Northern blot       | (153)      |
| Hyperthyroidism          | Rat      | No change in Nts mRNA| Medial Hypothalamus    | Northern blot       | (153)      |
| Hyperthyroidism          | Rat      | Lower Nts mRNA       | Anterior pituitary     | Northern blot       | (153)      |
| Schizophrenia            | Human    | Lower Nts            | CSF                    | RIA                 | (154)      |
| Stress-induced anorexia  | Mouse    | Higher activity of Nts-expressing neurons | Lateral septum | Photometry, optogenetics, and chemogenetics | (79) |
| Anorexia                 | Human    | NTS gene mutation    | Whole body             | Meta-analysis       | (24)       |
| Anorexia                 | Rat      | Higher Nts mRNA      | Retrochiasmatic area   | In situ analysis    | (91)       |

Summary of literature on central expression of Nts or xenin in diseases associated with altered body weight.

Abbreviations: ELISA, enzyme-linked immunosorbent assay; CSF, cerebrospinal fluid; LHA, lateral hypothalamic area; mRNA, messenger RNA; Nts, neurotensin; RIA, radioimmunoassay; SN, substantia nigra.
so may contribute to establishment of all dopamine circuits (124, 159). Lacking Nts-NtsR1 during development, as would be the case in individuals with loss-of-function mutations, could have profound organizational effects on the dopamine system that alter dopamine-mediated feeding behavior. This may explain why modulating Nts-NtsR1 constitutively vs in the established brain produces different effects on feeding behavior. Defining how the Nts system contributes to establishing dopamine pathways may reveal underpinnings of the disrupted circuitry, neurochemistry, and behavior in anorexia.

**Conclusion**

Nts exerts seemingly opposing effects on body weight via its fat-absorbing role in the intestine and anorectic effect in the brain. Viewed another way, these systems act in tandem to convey energy sufficiency, through metabolizing ingested fat via the intestine and acting via the brain to limit further intake of nutrients. One key question is whether dietary fat intake influences release of Nts from central Nts-expressing neurons, and if they are linked with the postigestive regulation of Nts in the periphery. Some central Nts neurons are regulated by hormonal signals of energy status, but they might be more important for long-term modulation of feeding and energy balance rather than meal-coupled regulators. Additionally, it will be vital to evaluate the roles of the elevated plasma Nts in obesity and after bariatric procedures: Are these increases an indicator of altered metabolic physiology or coincident markers of disfunction, and do they also invoke changes in central Nts signaling? Sustained elevations in plasma Nts might lead to chronic NtsR1/2 stimulation, leading to internalization of these GPCRs and diminished Nts-mediated effects. However, if elevated plasma Nts does not reach the deeper brain structures, it is possible that NtsR1/2 action might be preserved in these sites, and able to mediate anorectic actions. Given the growing view that bariatric procedures promote weight loss via altering the gut-brain axis, it is possible that some of the weight-reducing effects are mediated by biasing actions between peripheral and central Nts systems to promote central anorectic effects.

The next frontier of central Nts research is to define the function of specific central Nts circuits and determine whether any show bias for weight loss. Nts neurons within the hypothalamus and lateral septum have received the bulk of attention to date, but there may be other Nts-expressing populations that contribute to body weight in yet unrecognized ways. Similarly, it will be vital to systematically explore site- and cell-specific roles of NtsR1 and NtsR2 in modulating feeding and body weight. The role of NtsR2-expressing astrocytes is virtually untested but deserves attention in light of recent work showing that astrocytes and astrocyte-neuronal interactions profoundly regulate motivated behaviors. Defining the cells, circuits, and signaling mechanisms by which Nts modifies feeding and body weight is also critical to identify safe strategies to modulate energy balance. Thus, characterizing how the small Nts peptide acts in the brain to modify appetite could have big effects for understanding the development of disordered body weight and how to correct it.

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