Medial nucleus tractus solitarius oxytocin receptor signaling and food intake control: the role of gastrointestinal satiation signal processing

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Oxytocin (OT) is a nonapeptide synthesized in the paraventricular (PVH) and supraoptic nuclei (SON) of the hypothalamus. OT is well known for its effects on social and anxiety behaviors (5, 40), and accumulating evidence suggests a role for OT in energy balance control (44). Deficiencies in OT receptor (OT-R) signaling in humans (Prader Willi Syndrome) for OT in energy balance control (44). Deficiencies in OT receptor signaling and food intake control: the role of gastrointestinal satiation signal processing. Am J Physiol Regul Integr Comp Physiol 308: R800–R806, 2015. First published March 4, 2015; doi:10.1152/ajpregu.00534.2014.—Central oxytocin (OT) administration reduces food intake and its effects are mediated, in part, by hindbrain oxytocin receptor (OT-R) signaling. The neural substrate and mechanisms mediating the intake inhibitory effects of hindbrain OT-R signaling are undefined. We examined the hypothesis that hindbrain OT-R-mediated feeding inhibition results from an interaction between medial nucleus tractus solitarius (mNTS) OT-R signaling and the processing of gastrointestinal (GI) satiation signals by neurons of the mNTS. Here, we demonstrated that mNTS or fourth ventricle (4V) microinjections of OT in rats reduced chow intake in a dose-dependent manner. To examine whether the intake suppressive effects of mNTS OT-R signaling is mediated by GI signal processing, rats were injected with OT to the 4V (1 μg) or mNTS (0.3 μg), followed by self-ingestion of a nutrient preload, where each treatment was designed to be without effect on chow intake. Results showed that the combination of mNTS OT-R signaling and GI signaling processing by preload ingestion reduced chow intake significantly and to a greater extent than either stimulus alone. Using enzyme immunoassay, endogenous OT content in mNTS-enriched dorsal vagal complex (DVC) in response to ingestion of nutrient preload was measured. Results revealed that preload ingestion significantly elevated endogenous DVC OT content. Taken together, these findings provide evidence that mNTS neurons are a site of action for hindbrain OT-R signaling in food intake control and that the intake inhibitory effects of hindbrain mNTS OT-R signaling are mediated by interactions with GI satiation signal processing by mNTS neurons.

hindbrain; nucleus tractus solitarius; oxytocin; energy balance; satiation

OT-Rs are widely expressed throughout the brain including the PVH, arcuate nucleus of the hypothalamus (Arc), ventral mediod hypothalamus (VMH), ventral tegmental area (VTA), and medial nucleus tractus solitarius (mNTS) in the caudal hindbrain (19, 57, 62). Recent studies show that activation of OT-R in Arc (33), VMH (41), or VTA (38) reduce feeding. In addition to forebrain and midbrain regions, OT-R signaling in the hindbrain is also involved in energy balance control. Current evidence demonstrate that hindbrain fourth ventricle (4V) OT-R agonist delivery reduces food intake (22) and hindbrain blockade of endogenous OT-R signaling stimulates feeding (11, 12). The hindbrain neurons mediating the effects of 4V OT-R ligand delivery and the mechanistic basis for these feeding effects, however, remain undefined.

Neurons of the mNTS process energy status signals such as leptin, ghrelin, and glucagon-like peptide-1 (GLP-1) as well as vagally transmitted gastrointestinal (GI) satiation signals that each contribute critically to energy balance control (20). Several studies show that hindbrain OT-R signaling is downstream of central leptin receptor signaling (11, 35, 47) and that the intake inhibitory effects of leptin acting on mNTS neurons are mediated via interactions with GI afferent signal processing in the mNTS (21, 24, 27). It is possible that the intake inhibitory effects of OT-R signaling in the mNTS also involve interactions with the neural processing of GI satiation signals. Supporting this hypothesis are earlier studies showing that OT application excites gastric-stimulated neurons in the dorsal vagal complex (DVC) including the NTS and dorsal motor nucleus of the vagus (DMV) (36). More recently, rat hindbrain slice electrophysiological data from Peters and colleagues (47) showed that OT potentiates the excitatory effects of solitary tract electrical stimulation (a proxy for peripheral vagal afferent stimulation) on NTS neurons, and electron microscopic finding showed that NTS OT terminals make synaptic contacts with dendrites of mNTS neurons, suggesting a direct interaction between OT-R and GI vagal afferent signaling in the mNTS. Consistent with this perspective are anatomical data showing that OT-positive pPVN neuronal projections to NTS are located in close apposition to NTS neurons activated by the GI satiation peptide cholecystokinin (CCK) (9), as well as data showing that CCK activates pPVN OT neurons that project to the DVC (43). Complementing these structural data are results from behavioral studies showing that 4V delivery of OT-R antagonist or OT-R expressing neuron saporin lesions attenuates the intake inhibitory effects of CCK (4, 9).

While the literature suggests that hindbrain OT-R signaling reduces food intake via interactions with the processing of GI satiation signals, it is still unclear whether: 1) these effects are mediated specifically by mNTS OT-R signaling, 2) endogenous GI satiation signals interact with hindbrain/mNTS OT-R signaling to suppress intake, and 3) nutrient intake increases
DVC OT content. To address these points, experiments described here used behavioral/pharmacological and molecular assays to investigate the contribution of and mechanisms mediating hindbrain/mNTS OT-R signaling on food intake control. We found that targeted mNTS OT-R signaling reduced intake and that this effect was amplified when OT was given in combination with ingested nutrient/activation of GI satiation signals. Furthermore, DVC OT levels were significantly elevated in response to food intake. Collectively, these findings suggest that OT-R signaling in mNTS neurons interacts with the processing of endogenous GI satiation signals by these neurons to reduce food intake.

**METHODS**

**Animals**

Adult male Sprague-Dawley rats (250–265 g on arrival, Charles River Laboratories, Wilmington, MA) were individually housed in metal hanging cages under a 12-h light/12-h dark cycle (lights off at 11:00 AM). Rats had ad libitum access to pelleted chow (Purina 5001, St. Louis, MO) and water, unless otherwise stated. All procedures conformed to the institutional standards of the University of Pennsylvania Animal Care and Use Committee and were approved by the committee.

**Surgery**

Rats were anesthetized with intramuscular ketamine (90 mg/kg; Butler Animal Health Supply, Dublin, OH), xylazine (2.7 mg/kg; Anased, Shenandoah, IA), and acepromazine (0.64 mg/kg; Butler Animal Health Supply) followed by subcutaneous analgesia (2.0 mg/kg Loxicom; Midwest Veterinary Supply, Norristown, PA) post-surgery. Rats were implanted with unilateral guide cannulas (26 gauge; Plastics One, Roanoke, VA) with tip positioned 2 mm above the mNTS (coordinates: on midline, 2.5 mm anterior to occipital suture, 5.2 mm ventral to skull) or bilateral caudal mNTS [coordinates: ± 0.5 mm lateral to midline, 1.9 mm anterior to occipital suture, 6.8 mm ventral from skull, at a 15° angle (anterior to posterior)]. Injection tips that extend 2 mm below the cannula were used. Cannula placements were verified by assessing the sympathoadrenal-mediated glycaemic responses to 5-thio-D-glucose [4V: 210 mg/kg]. Cannula placements ventral from skull, at a 15° angle (anterior to posterior) and 5.2 mm ventral to skull were used. Injection sites were histologically verified postmortem. A representative image of the injection site at the mNTS is shown in Fig. 1.

**Experimental Procedures**

Rats were habituated to experimental procedures 7 days before experiments. All feeding experiments were conducted using within-subjects, counterbalanced design, with at least 48 h intervening experiments. All feeding experiments were conducted using within-subjects, counterbalanced design, with at least 48 h intervening experiments. Rats were implanted with unilateral guide cannulas (26 gauge; Plastics One, Roanoke, VA) with tip positioned 2 mm above the mNTS (coordinates: on midline, 2.5 mm anterior to occipital suture, 5.2 mm ventral to skull) or bilateral caudal mNTS [coordinates: ± 0.5 mm lateral to midline, 1.9 mm anterior to occipital suture, 6.8 mm ventral from skull, at a 15° angle (anterior to posterior)]. Injection tips that extend 2 mm below the cannula were used. Cannula placements were verified by assessing the sympathoadrenal-mediated glycaemic response to 5-thio-D-glucose [4V: 210 μg in 2 μl artificial cerebral spinal fluid (aCSF); mNTS: 24 μg in 100 nl aCSF] (51). A postinjection increase in blood glucose level of 100% or greater from baseline was required for subject inclusion. Cannula placement for mNTS was also histologically verified postmortem. A representative image of the injection site at the mNTS is shown in Fig. 1.

**Fig. 1.** Representative image of an injection site targeted at the medial nucleus tractus solitarius (mNTS). AP, area postrema; CC, central canal.

**Experiment 1: to determine the effects of hindbrain OT-R signaling on chow intake.** Rats (n = 12) received 4V OT injections [vehicle (Veh), 1 μg; 3 μg, 6 μg, 12 μg/1 μl] and chow intake (accounting for spillage) was manually measured 0.5, 1, 2, and 24 h postinjection. Body weight was also measured 24 h postinjection.

**Experiment 2: to assess the effects of mNTS OT-R signaling on chow intake.** Rats (n = 12) with mNTS cannulas received unilateral injections of OT (Veh, 0.3 μg, 1 μg/100 nl). The highest dose (1 μg) was determined in experiment 1 to be a ventricular subthreshold dose for chow intake effects. Chow intake and body weight were determined as stated in experiment 1.

**Experiment 3: to examine the intake effects of hindbrain OT-R antagonism on exogenous hindbrain OT administration.** To identify doses of the selective OT-R antagonist H-4928, which are subthreshold for effect when delivered to 4V, naïve rats (n = 14) were injected with H-4928 (Veh, 0.01 μg, 1 μg) to the 4V, and cumulative chow intake (accounting for spillage) was measured at 0.5, 1, and 2 h postinjection. With the use of a dose of H-4928 that was subthreshold for feeding effects (1 μg), rats (n = 13) received a 4V injection of H-4928, followed by 4V delivery of 3 μg OT. Cumulative chow intake (accounting for spillage) was determined at 0.5, 1, and 2 h postinjection.

**Experiment 4: to examine whether 4V or mNTS OT-R signaling interacts with the processing of GI satiation signals to reduce food intake.** To activate endogenous GI satiation signals, two different groups of rats (n = 14) with 4V or mNTS cannulas were trained to self-consume a fixed volume of vanilla-flavored Ensure (1.42 kcal/ml) (referred to as preload) within a 10-min period. Training was conducted for 7 days before experimental testing as follows. Rats were first given 24 h access to the preload to eliminate novelty. A preload of 12 ml was subsequently made available at dark cycle onset for 2–3 days until consumed to entirety within 10 min. To test the interaction between OT-R signaling and GI signal processing, a four-condition experiment involving a dose of OT and a volume of preload, both subthreshold for effects on food intake when given alone, was used as described previously (24, 27), resulting in four experimental conditions (Veh-no preload, Veh-preload, OT-no preload, OT-preload). During test days, food hoppers were removed 3 h before preload exposure to prevent chow intake that may affect the volume of preload consumed. Before dark onset, rats with 4V cannulas received either 1 μg OT or Veh, and rats with mNTS cannulas received either 0.3 μg OT or Veh. Rats were subsequently given either no preload or 7 ml preload (consumed within 10 min) 30 min after injection. Food hoppers were immediately returned after Ensure consumption and chow intake (accounting for spillage) determined at 0.5, 1, and 1.5 h.

**Experiment 5: to investigate the effects of food intake on DVC OT content.** To examine the relationship between DVC OT content and nutrient intake-induced activation of endogenous GI satiation signals,
postprandial DVC OT content was determined. In this experiment, a separate group of rats (n = 9) were trained to self-consume 12 ml preload (a volume determined in pilot studies to consistently reduce subsequent chow intake) within 10 min at onset of dark cycle. Food hoppers were removed 3 h before dark cycle onset on the experimental day to ensure similar baseline energy status between rats at dark cycle onset. Rats either had no preload or had access to 12 ml preload that was consumed to entirety within 10 min. Rats were lightly anesthetized with intramuscular ketamine (90 mg/kg), xylazine (2.7 mg/kg), and acepromazine (0.64 mg/kg) and decapitated 20 min after consumption. Brains were rapidly removed, flash frozen in cold isopentane, and stored in −80°C. DVC-enriched sections were isolated from 18 × 100 μm cryostat-cut frozen sections (Bregma −13.00 to −14.80), and OT content was determined using enzyme immunoassay. Briefly, DVC-enriched sections were boiled and homogenized in 50% acetic acid. Homogenate was centrifuged at 12,000 rpm for 20 min at 4°C and supernatant collected. Protein concentration was determined using BCA assay. Samples were then acidified and purified using C-18 Sep columns (200 mg). The eluted content was freeze-dried overnight. Lyophilized samples were reconstituted in assay buffer, and OT content was determined using an OT enzyme immunoassay kit (Phoenix Pharmaceuticals) according to manufacturer’s instructions. The sensitivity of the assay is 0.09 ng/ml with 100% specificity to human, rat, mouse, and bovine OT.

Statistics

Results are shown as means ± SE. Feeding behavior data were analyzed using repeated measures one-way or two-way ANOVA. When ANOVA identified a significant effect, Tukey’s Honestly Significant Difference (HSD) test was conducted post hoc. Data from DVC OT content were analyzed with Student’s unpaired t-test. All statistical analyses were conducted using Statistica software (Statsoft) and statistical significance was defined as P < 0.05.

RESULTS

Experiment 1: Hindbrain OT-R Signaling Reduced Chow Intake

OT delivered to the 4V reduced chow intake in a dose-dependent manner. Post hoc analyses indicated that 3, 6, and 12 μg, but not 1 μg, OT reduced chow intake at 0.5 h of dark cycle onset (P < 0.01). Chow intake remained significantly suppressed with 12 μg OT at 1 h (P < 0.05) (Fig. 2A). There was no effect of OT on 24 h food intake (Veh: 23.4 ± 0.9 g, 1 μg: 23.7 ± 0.6 g, 3 μg: 21.9 ± 0.8 g, 6 μg: 22.0 ± 0.8, 12 μg: 20.9 ± 1.7 g) or on body weight (Veh: −0.5 ± 1.1 g, 1 μg: −1.8 ± 1 g, 3 μg: −1.9 ± 0.9 g, 6 μg: −0.3 ± 1.9 g, 12 μg: −3.9 ± 3.5 g) compared with Veh treatment.

Experiment 2: mNTS OT-R Signaling Reduced Chow Intake

Direct mNTS delivery of 1 μg OT, a dose shown in experiment 1 to be without effect on intake (subthreshold) when delivered to the 4V, reduced chow intake at 0.5 h after onset of dark cycle (P < 0.05), whereas 0.3 μg OT did not significantly reduce chow intake (Fig. 2B). Similar to the data for 4V, mNTS OT delivery was without effect on 24 h food intake (Veh: 23.6 ± 1.2 g, 0.3 μg: 23.4 ± 1.6 g, 1 μg: 22.0 ± 1.5 g) or body weight (Veh: 0.3 ± 1.4 g, 0.3 μg: 1.45 ± 1.5 g, 1 μg: 1.6 ± 2.4 g) relative to Veh values.

Experiment 3: Hindbrain OT-R Antagonism Blocked the Intake Inhibitory Effects of Hindbrain OT-R Signaling

4V delivery of 0.01 or 1 μg OT-R antagonist H-4928 had no effect on feeding relative to Veh (Fig. 3A). To determine whether H-4928 attenuated the feeding inhibitory effects of OT in the hindbrain, the highest intake ineffective dose of H-4928 (1 μg) was delivered before 4V OT (3 μg) administration. Two-way repeated measures ANOVA revealed a significant main effect of OT (P < 0.05) and an interaction between OT and H-4928 (P < 0.05) at 0.5 and 1 h. 4V delivery of 3 μg OT alone reduced chow intake at 0.5 and 1 h (P < 0.01), When 1 μg H-4928 was administered before 3 μg OT delivery to the 4V, the intake inhibitory effects of OT were attenuated at 0.5 h (P < 0.05 H-4928/OT vs. Veh/Veh, P < 0.05 H-4928/OT vs. veh/OT) and completely blocked at 1 h (P < 0.05 Veh/OT vs. Veh/Veh, H-4928/Veh, H-4928/OT) (Fig. 3B).
Experiment 4: Combination of Hindbrain or mNTS OT-R Signaling and GI Satiation Signal Processing Amplifies the Intake Inhibitory Effect of Either Stimulus Alone

Two-way repeated measures ANOVA showed a significant main effect of preload ($P < 0.05$). Consistent with pilot testing results, 4V delivery of 1 µg OT (OT/no preload) or 7 ml preload (Veh/preload) alone did not reduce food intake at any time points measured. However, when rats received the combination of 1 µg OT and 7 ml preload (OT/preload), chow intake was significantly reduced at 0.5 h ($P < 0.05$ OT/preload vs. Veh/no preload, OT/no preload, Veh/preload) (Fig. 4A).

In a separate group of naïve rats with mNTS cannulas, two-way repeated measures ANOVA revealed a significant main effect of OT and preload ($P < 0.05$). While 0.3 µg OT and 7 ml preload treatments were selected on the basis of our pilot data to be without effect, they each significantly reduced chow intake at 0.5 h. Nevertheless, the combinatorial treatment of OT and preload (OT/preload) resulted in a significantly greater suppression of intake compared with either treatment alone: OT/no preload or Veh/preload or Veh/no preload at 0.5 h ($P < 0.05$). Chow intake remained significantly reduced in the OT/preload group at 1 h ($P < 0.05$ OT/preload vs. Veh/no preload, OT/no preload) and 1.5 h ($P < 0.05$ OT/preload vs. Veh/no preload, OT/no preload, Veh/preload) (Fig. 4B).

Experiment 5: Food Intake Increased OT Content of the DVC

OT content of the DVC in rats that consumed 12 ml preload was significantly higher (22.5%) than control rats that received no preload ($P < 0.05$) (Fig. 5).

DISCUSSION

Previous studies show that hindbrain OT-R signaling contributes to the intake inhibitory effects of central OT-R signaling. Unspecified, however, were the neural substrates and mechanisms mediating the food intake suppressive effects of hindbrain ventricular OT delivery. Here, we showed that 1) OT-R signaling in mNTS neurons reduced food intake, and 2) the intake inhibitory effects of mNTS OT-R signaling were amplified by local processing of GI satiation signals such that the combined intake suppression was greater than the effects of either stimulus presented alone. The present study also established for the first time that food intake increased endogenous OT content in the DVC. This work therefore provides evidence that the combination of OT-R signaling and GI satiation signal processing by hindbrain mNTS neurons contributes to the food intake inhibitory effects of hindbrain OT-R signaling.

It is well-established that neurons in the mNTS are critical for energy balance control (20). Here, we demonstrated that OT-R signaling in mNTS neurons reduced food intake and contributes in part to the intake inhibitory effects of 4V OT administration. To address whether the intake inhibitory effects of NTS OT-R stimulation are mediated by interacting with NTS vagal afferent processing of GI satiation signals, we examined 1) the combined effects of exogenous NTS OT delivery and endogenous GI satiation signals (activated by a preload) on food intake, and 2) the effect of feeding-driven activation of endogenous GI satiation signaling on endogenous DVC OT content. Results revealed that the combination of NTS OT delivery and the stimulation of endogenous GI satiation signals significantly reduced food intake, an effect that exceeded the intake inhibitory effects of either stimulus when presented alone (a pattern similar to that when OT was delivered to the 4V). These results provide a functional framework for slice electrophysiological data from a seminal study by Peters and colleagues (47) who also demonstrated a combinatorial effect of OT and solitary tract electric stimulation on NTS neuron activation. They showed that OT bath application to hindbrain slices increases the excitation of stimulated solitary tract neurons (a proxy for vagal afferent nerve stimulation) through enhanced presynaptic glutamate release and depolarization of postsynaptic mNTS neurons (47). In addition, McCann and Rogers (36) also showed that a proportion of DVC neurons that are stimulated by OT are in fact, GI related. Together, these studies indicate an interaction between OT-R signaling and GI signal processing that occurs in the mNTS.

To link GI satiation signaling and its central processing to hindbrain mNTS OT-R signaling, we further explored whether food ingestion elevates endogenous OT content in mNTS-
enriched-DVC tissue. Consumption of the nutrient complete liquid diet Ensure (referred to as preload) was selected as a strategy to engage a range of endogenous GI satiation signals, e.g., gastric distension, CCK, GLP-1, PYY 3–36 secretion, and intestinal nutrient signaling. Self-ingestion of 12 ml preload significantly increased OT content in the DVC compared with rats that did not consume the preload. To our knowledge, this is the first time OT content is measured in the DVC following feeding. Previous studies reported increases in plasma OT levels (58) and PVH OT mRNA (28) after food intake and a refeed (following a fast), respectively. Present findings therefore highlight the DVC as a novel site for food intake/GI signals-driven increase in OT levels and suggest that DVC OT-R signaling is part of a homeostatic circuit involved in food intake control.

While using nutrient ingestion (the preload) physiologically examines the effects of multiple endogenous GI satiation signal activation, the role of particular GI signals contributing to the feeding effects of mNTS OT-R signaling is unclear. Blevins and colleagues (9) demonstrated that NTS OT fibers closely appose CCK-activated NTS neurons, suggesting a potential interaction between CCK and OT-R signaling in the mNTS in food intake control. In support of the anatomical data, they showed that hindbrain OT-R blockade attenuates CCK-induced cFos immunoreactivity in the NTS and, more importantly, blocked the intake inhibitory effects of CCK (9). Saporin ablation of OT-R expressing neurons in the NTS also attenuated the intake inhibitory effects of CCK (4). These findings are in line with results from the present study and suggest that CCK is one of the GI satiation signals that plays a role in the intake inhibitory effects of hindbrain OT-R signaling. Additionally, previous studies show that plasma OT, which also contributes to intake reduction, is increased in response to food intake (58), CCK administration (58, 61), gastric distention (39), and changes in plasma osmolality (induced by NaCl or mannitol injection) (7, 8). Whether gastric distention and plasma osmolality contributes to mNTS OT-R signaling-induced intake reduction and/or lead to elevated DVC OT content remains to be determined.

The neural mechanisms mediating the increase in DVC OT content in response to food intake are also not well understood. Nevertheless, the increase in PVH OT levels following GI signal activation/food intake is thought to be mediated via the activation of vagal afferent neurons that engage PVH projecting A2 noradrenergic neurons in the caudal medulla to stimulate PVH OT synthesis (45). Activation of magnocellular OT neurons by peripheral CCK, on the other hand, increases plasma OT release via the hypophyseal system (58). Given that PVH electrical stimulation increases DVC OT release (29) and that DVC receives OT projections solely from the pPVH (48), it is possible that feeding-stimulated GI satiation signals increase vagal afferent neurotransmission and activate medullary A2 neurons that project to the PVH, which in turn activate PVH OT neurons to increase OT synthesis, resulting in increased DVC OT content. In line with this hypothesis are studies showing that PVH OT mRNA is increased with refeeding (28) and reduced following a fast (46), indicating that central OT production is sensitive to the activation and inhibition of GI signals (refeeding and fasting, respectively). However, transcriptional events typically take hours to weeks to occur. Since the increase in DVC OT content was observed 30 min after preload consumption, it is unlikely that the elevated DVC OT content is a result of increased OT synthesis in the pPVH. This suggests that the rise in DVC OT content likely occurred via increased transport of matured OT vesicles from pPVH to DVC and released in response to vagal afferent stimulation (18). To our knowledge, the synaptic machinery governing OT release in the DVC is still unknown. In the hypothalamus, synaptic OT release is negatively regulated by synaptotagmin-4, a synaptic exocytosis modulator (64). Furthermore, melanocortin stimulation of hypothalamic OT neurons stimulates OT release by mobilizing intracellular calcium stores (53). Whether synaptotagmin-4 function and/or modulation of intracellular calcium stores is relevant in GI signal-stimulated DVC OT release is unclear and are important directions to pursue to understand the cellular and molecular mechanisms of DVC OT release. In the present study, DVC OT content was measured with enzyme immunoassay. This method does not differentiate between presynaptic and postsynaptic OT levels. Nevertheless, that the half-life of OT in the brain is ~30 min (18) and that rats were euthanized 30 min after preload, it is likely that the OT content measured in the present study also includes postsynaptic OT release in the DVC. Microdialysis studies will be required to confirm that food intake specifically increases DVC OT release.

While experiments here show that mNTS OT-R signaling and GI signal processing combine to reduce food intake, it is unclear whether the integration of both signals occurs within the same neuron or via a common intracellular signaling pathway. Future experiments are required to address this question. It is also important to note that neurons in the DMV also express OT-R (19, 57) and their dendrites extend to the NTS (49). Moreover, OT stimulation of DMV contributes to feeding behavior (16, 52). Therefore, we cannot exclude the possibility for a role of DMV in the interaction between OT-R signaling and GI signal processing in food intake control. This hypothesis remains to be directly examined.

We acknowledge that the duration of the intake inhibitory effects of 4V or mNTS OT is short (up to 1 h). Although this is in contrast to forebrain ventricular OT effects, which lasts for at least 6 h postinjection in nonfood-deprived rats (34, 63), it is consistent with other reports of 4V or peripheral OT delivery (22). This difference in the effect duration suggests the contribution of specific forebrain nuclei in mediating the longer lasting feeding effect, for example, the VMH where OT delivery reduces food intake for at least 4 h (41). PVH and SON (17) neurons are also activated after meal initiation (26) and process a positive feedback mechanism where OT stimulates local dendritic OT release (30), which in the context of feeding control, may increase hypothalamic (VMH, PVH, SON) OT-R signaling and prolong the duration of feeding effects. The short duration of effects of hindbrain mNTS OT-R signaling, on the other hand, suggests that OT in the hindbrain mNTS acts as a short-term signal to reduce food intake. We also noticed that the baseline chow intake in the present study appears to be slightly different between experiments. These differences may arise from the variation in time that food was removed and returned before and after injections, respectively (30 min vs. 2 h).

Current data show intake effects of mNTS OT-R signaling that are consistent with previous research in rats. However, studies in mice report limited or no effect of OT-R signaling on feeding behavior (13, 54, 56, 59). One study in mice reported
very few pPVH OT neurons that project to the mNTS and suggested that OT immunoreactivity observed in the NTS are OT fibers of passage and not neuronal terminals. In contrast, studies in rats using injection of retrograde neuronal tracer to the NTS show colabeled tracer in pPVH OT neurons, thus suggesting that a population of pPVH OT neurons projects to the NTS (46, 48). Furthermore, electron microscopic data show that OT-positive terminals make synaptic contact with dendrites in the NTS (47). These conflicting findings raise the possibility of species (rat vs. mouse) differences in the anatomical and functional aspects of central OT-R signaling, a phenomenon that is also reported for hindbrain leptin receptor and proglucagon (GLP-1 expressing) neurons (23). Future studies are therefore warranted to provide a more detailed comparison of central OT-R signaling pathways and the interaction with GI satiation signals between rats and mice.

**Perspectives and Significance**

The present study showed for the first time that the intake inhibitory effects of hindbrain OT-R signaling occur at the level of the mNTS via the interactions with GI satiation signal processing. We also provided novel evidence that food intake increases DVC OT content. It is therefore proposed that mNTS OT-R signaling and its interaction with GI signal processing are involved in a homeostatic circuit of feeding control whereby the rise in endogenous DVC OT level following food intake amplifies the intake inhibitory effects of GI satiation signals, leading to a reduction in food intake or meal termination. Future studies should examine the molecular and cellular aspects of DVC OT release to better understand the mechanisms underlying the intake inhibitory effects mNTS OTR signaling and its interaction with GI satiation signal processing.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**


**REFERENCES**


Sutton AK, Pei H, Burnett KH, Myers MG Jr, Rhodes CJ, Olson DP. Control of food intake and energy expenditure by nes1 neurons of the paraventricular hypothalms. J Neurosci 34: 15306–15318, 2014.


