Putative roles of neuropeptides in vagal afferent signaling

Guillaume de Lartigue[*]

Dept Anatomy, Physiology and Cell Biology, UC Davis School of Veterinary Medicine, Davis, CA 95616, USA

Abstract

The vagus nerve is a major pathway by which information is communicated between the brain and peripheral organs. Sensory neurons of the vagus are located in the nodose ganglia. These vagal afferent neurons innervate the heart, the lung and the gastrointestinal tract, and convey information about peripheral signals to the brain important in the control of cardiovascular tone, respiratory tone, and satiation, respectively. Glutamate is thought to be the primary neurotransmitter involved in conveying all of this information to the brain. It remains unclear how a single neurotransmitter can regulate such an extensive list of physiological functions from a wide range of visceral sites. Many neurotransmitters have been identified in vagal afferent neurons and have been suggested to modulate the physiological functions of glutamate. Specifically, the anorectic peptide transmitters, cocaine and amphetamine regulated transcript (CART) and the orexigenic peptide transmitters, melanin concentrating hormone (MCH) are differentially regulated in vagal afferent neurons and have opposing effects on food intake. Using these two peptides as a model, this review will discuss the potential role of peptide transmitters in providing a more precise and refined modulatory control of the broad physiological functions of glutamate, especially in relation to the control of feeding.

1. Introduction

The vagus nerve provides a major bidirectional route of communication between the brain and peripheral organs. Afferent fibers of the vagus nerve convey sensory information from the gastrointestinal tract, heart, lung, liver, and pancreas to the central nervous system; while vagal efferent fibers are involved in motor function conveyed from the CNS to visceral organs. The pseudounipolar cell bodies of vagal afferents reside in bilateral nodose ganglia, and are involved in sensing multiple mechanical, chemical, osmotic, thermal, and possibly nociceptive stimuli. The total number of vagal afferent neurons has been found to be roughly similar across a number of different species and ranges from 16,000 to 18,000 perikarya, with the densest distribution of peripherally projecting fibers innervating the proximal GI

[*] Vet Med: APC, 2017 VM3B, 1 Shields Ave, UC Davis, Davis, CA 95616, USA. Tel.: +1 530 754 0605; fax: +1 530 752 7690. gdelartigue@ucdavis.edu..

tract. Tracing experiments demonstrate that the right and left nodose ganglia innervate slightly different peripheral sites [1,2]. Fibers of vagal afferent neurons also project to CNS neurons in the brain stem where they make synaptic connections with individual second order neurons in the nucleus of the solitary tract (NTS).

As a result of the large quantity of information transmitted by vagal afferent fibers it is unsurprising that they outnumber the vagal efferent fibers by approximately 9 to 1 [3]. Vagal efferent neurons carry parasym-pathetic motor-control fibers that are associated with the autonomic nervous system from the brainstem to visceral organs like the lungs, the heart, and the gastrointestinal tract. Vagal motor neurons are localized in two brain stem nuclei in the nucleus ambiguous (NA) and in the dorsal motor nucleus. They receive afferent inputs predominantly from NTS neurons, either as fiber projections from the NTS neurons or by sending their own dendrites into the NTS [4].

The vast array of information sensed by the vagus nerve from a large number of peripheral organs results in an extensive range of physiological functions, including a role in cardiovascular tone, respiratory tone, satiation, GI motility, digestion and absorption, emesis, and inflammation. The mechanism by which this information is communicated to the brain is not well defined. The release of the neurotransmitter glutamate from vagal afferent neurons has been demonstrated to play a role in a large number of these physiological functions, but the mechanisms by which this single neurotransmitter can regulate such an extensive list of physiological functions from a wide range of visceral sites remain unclear. The mechanisms of glutamate signaling will be addressed in this review. Extensive studies of the late 80s and 90s demonstrated that vagal afferent neurons express a range of peptides that can play a role in mediating some of the functions associated with vagal afferent neurons. Recently two novel putative peptide transmitters have been identified in vagal afferent neurons. These peptides are differentially expressed in vagal afferent neurons and are associated with opposing effects on feeding behavior. Using these two peptides as a model, this review will discuss the potential role of peptide transmitters in providing a more precise and refined modulatory control of the broad physiological functions of glutamate, especially in relation to the control of feeding.

2. Evidence for a role of glutamate in vagal afferent signaling in the NTS

The view that glutamate is the primary neurotransmitter of vagal afferent signaling to the NTS is widely shared [5–10]. This view is grounded in substantial anatomical, electrophysiological, and pharmacological evidence. Glutamate immunoreactivity can be observed in approximately 60% of neuronal cell bodies in the nodose ganglion [11–13], as well as axons in the tractus solitarius and terminals in the NTS [12]. Nodosectomy, which removes the vagal afferent cell bodies, results in a 40% reduction in the high-affinity uptake of glutamate in the medial NTS of rats [14]. Furthermore, immunohistochemical data suggests that both types of vesicular glutamate transporter, VGLUT1 and VGLUT2, required for packaging cytoplasmic glutamate into vesicles, are present in vagal fibers and terminals in the NTS since they are significantly reduced following nodose ganglionectomy [15]. Interestingly, vagal afferents fibers retrogradely labeled from the stomach are predominantly VGLUT2 positive, while fibers retrogradely labeled from the heart are

VGLUT1 positive [16]. In summary, the machinery required for uptake and packaging of glutamate is present in vagal afferent neurons.

Indirect evidence for a signaling role of glutamate in the NTS was demonstrated by intracellular recordings in slices of rat brain. Postsynaptic potentials evoked in NTS neurons were identified as predominantly excitatory [17]. Furthermore, microinjection of [3H]-D-aspartate, a biochemical marker for excitatory amino acid-utilizing neurons, into the NTS of rats, retrogradely labels to cell bodies of nodose ganglia neurons [18]. Direct measurement of glutamate concentration in the NTS by microdialysis demonstrated that glutamate levels increased in the NTS following electrical [19,20], chemical [11,21], and mechanical stimulation of the vagal afferent fibers [22]. Unilateral nodosectomy reduced NTS levels of glutamate on the ipsilateral, but not the contralateral [23,24]. Together these data suggest that many of the vagal afferent fibers projecting to the NTS store and release glutamate, consistent with a transmitter role for glutamate. Although it should be noted that at least one group repeatedly failed to demonstrate changes in glutamate concentration in the NTS by microdialysis in response to vagal afferent electrical stimulation or nodosectomy [25–27].

Importantly, the receptors required for glutamate signaling can be found on postsynaptic NTS neurons. Over the years, molecular cloning of cDNA encoding glutamate receptors has revealed multiple groups of ionotropic glutamate receptor subunits [28]. These included six *N*-methyl-D-aspartate (NMDA) receptor subunits (NR1, NR2A to NR2D, and NR3), four α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) receptor subunits (GluR1–GluR4), and five kainate (KA) receptor subunits (GluR5 to GluR7, KA1, and KA2). Multiple G-protein-coupled receptors (GPCR), known as metabotropic glutamate receptors, have also been identified [29]. All of these receptors can be found in the NTS [30–32]. Both microinjection of glutamate into the NTS and vagal afferent stimulation can induce a wide range of physiological responses and these effects can be blocked using glutamate receptor antagonists [22,33–36]. Therefore there is substantial evidence supporting the concept that glutamate is a major neurotransmitter of vagal afferent neurons in the NTS that mediates physiological responses to peripheral sensory information (see Fig. 1).

3. Evidence that glutamate is involved in feeding

Bednar et al. [37] were the first to provide evidence that glutamate release from vagal afferent fibers in the NTS could mediate feeding behavior. They found that in micropunches of the medial NTS, fasted rats had reduced glutamate concentrations; while intraperitoneal (IP) injections of the gastrointestinal hormone cholecystokinin (CCK) or ingestion of sucrose reinstated glutamate levels. Furthermore they reported that IP injection of the non-competitive NMDA receptor antagonist, MK801, facilitated sucrose ingestion and antagonized CCK-induced satiation. Ritter and Burns published a series of papers expanding on the idea that NMDA receptors mediate vagally-induced ingestion. They demonstrated that systemic NMDA receptor blockade increased intake of solid food or 15% sucrose solutions after an overnight fast, without affecting water intake in rats [38]. Intake of 0.2% saccharin solution, which has no nutritive benefit, was not increased with MK801 administration [39]. They reported that IP MK801 did not cause meal initiation, but rather increased the size and duration of meals that were already initiated [39]. Most convincingly

they demonstrated that subdiaphragmatic vagotomy prevented IP MK-801-induced increases in meal duration [40], suggesting that glutamate activation of NMDA receptors mediates vagal transmission of satiety signals from the GI tract.

A number of findings that followed this work brought into questioned the site of action of systemic MK801. It became clear that IP MK801 did not inhibit satiation caused by vagal afferent glutamate activation of NTS neurons, but rather caused increased food intake by inhibiting activation of vagal efferent neurons leading to acceleration of gastric emptying. Two separate studies demonstrated that the stomach was important in mediating the effects of systemic MK801. Pretreatment of IP MK-801 failed to attenuate satiation of macronutrients in which the stomach was bypassed by infusion into the intestine [41] and also failed to prevent the satiating effects of exogenous CCK, known to be released from enteroendocrine I cells predominantly located in the duodenum [42]. It was then found that inducing vagal afferent lesions with capsaicin failed to prevent MK801 induced increases in meal duration; while inhibiting both vagal afferent and efferent signaling, by either subdiaphragmatic vagotomy [40,43] or lesioning the dorsal vagal complex [44], blocked the effects of systemic MK801. This suggested that vagal efferent neurons were the target site of IP MK801 (see Fig. 2). Finally, it was demonstrated that IP injection of MK801 acting on vagal efferent neurons accelerated gastric emptying [45,46].

Crucially endogenous glutamate was demonstrated to be released from vagal afferent neurons and inhibit food intake by activating postsynaptic NTS neurons. Firstly Covasa *et al*. demonstrated that 4th ventricle injection of MK801 increased food intake through a mechanism independent of gastric emptying [47]. Multiple studies subsequently reported that microinjection of NMDA receptor antagonists into the medial NTS of fasted rats increased meal size [48–53], providing evidence that at the site of vagal afferent fiber termination, endogenous glutamate was present and involved in satiation. Interestingly cannula tips located tenths of millimeters outside of this region failed to increase feeding [48]. MK801 has been reported to have off-target effects, interacting with other ion channels; however the NMDA receptor antagonists, AP5, and a NR2 subunit antagonist that distinguishes between different types of NMDA receptors, have also been shown to increase food intake in rats when injected into the medial NTS [49,50]. CCK-induced satiation, known to activate a vagal afferent pathway, was also found to be abolished by NTS injection of MK801 [51]. Neuro-anatomical studies demonstrated that exogenous CCK administration activated neurons in the medial NTS, and that activation of these neurons was abolished with NMDA receptor antagonists [51,53]. Importantly, unlike systemic administration of MK801, the orexigenic effects of intraNTS MK801 were not inhibited by subnodose vagotomy. Taken together with findings that unilateral nodosectomy prevented increased meal size of ipsilateral, but not contralateral, injection of MK801 into the medial NTS [52], this conclusively demonstrated that vagal afferent fibers release of endogenous glutamate activates NMDA receptors in the medial NTS to modulate satiation (see Fig. 2).

The role of non-NMDA receptor of the hindbrain in the control of food intake is less clear. Anatomical studies indicate that most if not all NTS neurons express non-NMDA receptors [54]. Although, gastric distension or duodenal nutrient stimuli activate NTS neurons that express the AMPA receptors, GLUR2/3 [55], pharmacological studies have failed to

demonstrate a role for non-NMDA receptors in satiation. Inhibiting both AMPA receptor and kainite receptors in the hindbrain using the antagonist, 1,2,3,4-tetrahydro-6-nitro-2,3-dioxobenzo[*f*] quinoxaline-7-sulfonamide (NBQX), was found to potently suppress sucrose, chow, and water intake in a dose-dependent manner in both fed or fasted rats. In this thorough study the authors demonstrate that NBQX injection into the NTS does not cause conditioned taste aversion or motor deficiency [55]. Instead the authors suggest that reduced sense of taste, caused by NBQX inhibiting taste-induced activity of rostral NTS neurons, could be responsible for the reduction in licking frequency. Although from these studies it cannot be ruled out that endogenous glutamate activation of AMPA receptor results in increased food intake. Irrespective of the mechanisms by which intraNTS NBQX inhibits food intake, the data indicates that despite virtually all NTS neurons expressing non-NMDA receptors, these are not involved in mediating the satiating effects of vagal afferents from the gastrointestinal tract. Instead endogenous glutamate in the NTS requires NMDA receptors to mediate satiation.

4. Other physiological functions mediated by glutamate

As alluded to above, vagal afferent fibers convey an impressive array of information to the NTS. Vagal afferent fibers innervate the oral cavity, esophagus, stomach, small intestine, liver, pancreas, heart, and lungs and are therefore involved in a number of different physiological functions. There is a large body of evidence suggesting that glutamate is the primary neurotransmitter released from vagal afferent terminals to modulate respiration (see [56] for review), cardiovascular reflexes (see [57] and [58] for review), GI motility (see [6] for review), ingestion (see [4] for review) as well as provide information to the brain about the types (lipid, carbohydrate, and protein), quantity, and location of these nutrients within the gastrointestinal tract resulting in satiation, and may also be involved in taste perception [59] (see Table 1). This is a very broad and extensive range of information to be conveyed to the brain by one neurotransmitter. How can all of this information be differentially relayed to the brain by glutamate?

This question has not been directly addressed in the literature, mostly because it is not currently possible to determine the functional role of individual neurons in the NTS. Despite the lack of strong empirical evidence, the prevailing view is that fibers projecting from specific visceral sites activate precise neurons in the NTS with individual function. For example, vagal afferent fibers innervating the gut will sense distension of the stomach, send an action potential along the axon which releases glutamate onto a postsynaptic NTS neuron involved in motility, which will control gastric emptying by activating the vago-vagal reflex. Therefore according to this hypothesis it is important that the correct neuron is activated to prevent inappropriate physiological response requiring both tight spatial and temporal control of glutamate release. How does this occur?

As the prototypical fast neurotransmitter, glutamate is transiently released in short bursts from afferent fibers following depolarization caused by peripheral stimuli [60]. A negative feedback system, by which glutamate activates metabotropic glutamate receptors on the presynaptic cleft, prevents prolonged release of glutamate [61]. In addition, glutamate is rapidly removed from the synapse by glutamate transporters predominantly found on glial

cells that surround the synapse [62]. This process is highly efficient since it happens against the electrochemical gradient and allows glutamate to be recycled [62]. Most importantly the density of these transporters can control the concentration of glutamate in the synapse, the length of time glutamate is available to bind to ionotropic glutamate receptors on the post-synaptic cleft, and reduce glutamate diffusion out of the synapse, thereby limiting activation of neighboring neurons. Thus, at least in theory, tight spatial and temporal control of glutamate release from fibers projecting from specific visceral sites can result in precise activation of individual neurons in the NTS to mediate a specific physiological response.

There is some evidence for viscerotopic distribution in the NTS [63–66]. This is especially true of the gastrointestinal tract, with rostrocaudal organization in nodose ganglia neurons and the NTS mapping the length of the alimentary tract [64]. As food travels through the alimentary tract, more caudal regions of the NTS are activated [66]. Certainly this may provide some insight as to how subdiaphragmatic vagal afferents can provide information about the location of the nutrients within the gastrointestinal tract to the brain. However, there is no clear pattern of organization for vagal afferent fibers projecting from different visceral afferent regions; afferents innervating the lung, heart and gut are all bundled together and terminate in similar sub-regions of the NTS [65]. Furthermore, there does not seem to be viscerotopic distribution of postsynaptic NTS neurons with different functions. Neurons receiving different sensory inputs from the subdiaphragmatic vagus nerve, baroreceptors or chemoreceptors all localize within the same sub-region of the NTS [67]. For this reason the prevailing view is that fibers projecting from specific visceral sites activate precise neurons in the NTS [68]. This would explain how the same neurotransmitter can produce different physiological responses to peripheral signals in the NTS and provide a wide range of information about the nutritional content of food and its transit through the gastrointestinal tract. Further work is still required to determine the mechanisms by which fibers from nodose ganglia neurons project to individual neurons in the NTS during development in light of the seeming lack of organization of these second order neurons. Methodical mapping of these neurons within the NTS may provide some insight.

5. Rationale for a role of other neurotransmitters

There are three flaws that exist with the concept of glutamate as the predominant neurotransmitter of vagal afferent neurons:

1. NMDA receptors require a depolarizing event in addition to glutamate in order to be activated, and therefore glutamate alone is insufficient to regulate satiation.

2. Since NTS neurons with different functions are intermingled in the same regions, any spillover of glutamate out of the synapse would cause inappropriate functional responses to peripheral signals

3. Meal size and duration vary greatly across the day even when the same food with the same nutrient content is ingested, which suggests that a greater plasticity of signaling is required than can be provided by glutamate alone.

In this section each of these points will be addressed in more detail and the rationale for the involvement of modulatory peptides released from vagal afferent alongside glutamate will be discussed as a requirement for appropriate glutamate signaling.

1. Glutamate binding to NMDA alone is insufficient to activate neurons. At resting membrane potential, NMDA receptors are blocked by magnesium ions; an additional membrane depolarization event is required for the Mg^{2+} block to be relieved and allow those NMDA receptors which have glutamate bound to open [69]. The classical view is that glutamate acting on non-NMDA receptors would depolarize the neuron and enable glutamate activation of NMDA receptors. However, as discussed above, non-NMDA receptor antagonists are not involved in satiation. Therefore glutamate alone is insufficient to result in satiation and something else is required to depolarize the postsynaptic NTS neurons. Modulatory neuropep-tides co-released with glutamate from vagal afferent fibers provide an elegant solution to this problem. These peptides could be released alongside glutamate in response to a specific peripheral stimulus so that they can 1) modulate glutamate-induced NMDA activation by modifying the membrane potential, and 2) target the intended postsynaptic neuron, without activating neighboring quiescent neurons that lack the receptor for the neuropeptide. The presence of inhibitory and stimulatory modulatory peptides could have opposing effects on the membrane potential of postsynaptic neurons and either prevent or enable NMDA activation in the presence of glutamate. It is also possible that modulatory peptides regulate non-NMDA receptors, which would reconcile the anatomical evidence that most NTS neurons express these receptors, with the pharmacological data suggesting that they are not involved in satiation.

2. Despite the very high numbers of glutamate transporters surrounding synapses, evidence has emerged that glutamate can leak out from its synapse and diffuse to activate receptors on inactive neighboring synapses [70]. This process, known as spillover, may be problematic in the NTS where multiple neurons with different function intermingle. Spillover has predominantly been identified in hippocampal slices, however glutamate spillover in the NTS has been hypothesized in the past [54,71]. Modulatory peptides released from vagal afferent neurons alongside glutamate could prevent unwanted side-effects of spillover onto neighboring neurons, by depolarizing only the subset of NTS neurons that encode the appropriate neuropeptide transmitter.

3. Finally meal patterns in ad *libitum* fed animals are highly variable even in response to the same nutrient composition. Ingestion of the same chow diet (or any predefined nutrient composition) should result in the same release of glutamate in response to nutrient activation of taste receptors, swallowing, gastric distension, release of gastrointestinal hormones and absorption, resulting in the same amount of satiation. So every meal should terminate at the same time. However, this is not the case, instead each meal is heterogeneous. These observations suggest a certain amount of plasticity to gut–brain signaling must occur and this cannot be attained from glutamate alone. Instead plasticity in the expression and release of modulatory peptides could address this problem.

The advantage of neuromodulators participating in glutamate activation in the NTS is that they can be released alongside glutamate from vagal afferent fibers in response to the same visceral stimulus, and provide close regulation of glutamate-induced effects. Modulatory peptide transmitters could regulate glutamate activation of NTS neurons in multiple ways. Presynaptically, modulatory neurotransmitters could control the duration of glutamate secretion from vagal afferent neurons. Since neuropeptide transmitters remain in the synapse longer than glutamate, this could even allow them to affect the release of glutamate in response to future peripheral stimuli. Post-synaptically, they could alter the membrane potential of postsynaptic NTS neurons to control glutamate-induced activation of NMDA receptors. These modulatory peptides could alter glutamate transporter expression on glial cells in the NTS to reduce/increase glutamate re-uptake to change the duration and site of action of glutamate. Finally, they could bind neighboring quiescent neurons to regulate the convergence of different physiological processes. This would enable a primary site of crosstalk across multiple organ systems. For example, *Gilbey et al*. have demonstrated that there is a significant overlap in respiration and cardiovascular signaling to maintain an appropriate balance of oxygen and carbon dioxide in response to changes in physiological demand [72]. It could be possible for peptides to induce all or some of these modulatory effects at the same time, or for one peptide to modulate one aspect of glutamate signaling while another peptide to modulate a different aspect.

Neuropeptides are well suited to a modulatory role because they have slow, prolonged effects compared to classical neurotransmitters. While glutamate can be rapidly removed from the synapse, no evidence of peptide re-uptake has been observed [73]. Instead, neuropeptides are slowly removed from the extracellular space by diffusion and breakdown by proteases [73]; some neuropeptides have been shown to have half-lives of 20 min in the brain [74]. Neuropeptide transmitters also activate GPCR, which produce slower signals than fast synaptic transmission. Unlike glutamate, which is recycled at the synapse, neuropeptides are synthesized in the nodose ganglia and are transported by fast axonal transport [73]. There is evidence that peptide levels may vary considerably in response to different stimuli [73], this makes them ideal modulators of fast neurotransmission. Unsurprisingly, neuropep-tides are found in different vesicles to classical neurotransmitters. Neuropeptides are found in sparse large dense core vesicles, while classical neurotransmitters are contained within highly abundant small synaptic vesicles that occupy a large portion of the axon terminal [75,76]. It is unclear whether these vesicles are released together or separately; although, there is evidence that these can be differentially released [77]. It was reported that neuropeptides in large dense core vesicles were predominantly released outside of the synapse [78,79]. However evidence exists to support the idea that neuropeptides are also released at synapses [80]. More recently, using novel fluorescent probes, it has been found that although dense core vesicles were not enriched in synaptic terminals, they were preferentially released at the synapse [81]. In this study, it was further demonstrated that the Munc13 family of proteins, essential for the assembly and release of classical neurotransmitters from synaptic vesicles, also facilitate secretion of neuropeptides at synaptic terminals [81].

Although neuropeptides are preferentially released at the synapse, they are clearly also released extrasynaptically. We can only speculate the reason for extrasynaptic release of neuropeptides. Perhaps this enables neuropeptides to increase the area over which they can exert their effects [78]. Extrasynaptic release would blanket a larger area in the NTS than glutamate while only activating those NTS neurons that were coded with the appropriate peptide receptors. This would be beneficial as it could modulate glutamate release from neighboring presynaptic vagal afferents, enable modulation of postsynaptic gluta-mate activation prior to the complete transport of neuropeptide from the cell body to the synapse, and/or enable coordinated activation of multiple postsynaptic neurons coded with the peptide receptor. Understanding the signals involved in promoting synaptic or extrasynaptic release of neuropeptide may provide insight into the functional importance of these different mechanisms.

6. Evidence for a role of neuropeptides in satiation

The idea of neuromodulators is supported by the fact that multiple peptides are expressed in nodose ganglia neurons (see Table 1). Immunoreactivity in NTS fibers for all of these peptides is significantly reduced by vagal ablation or nodosectomy and receptors for these peptides have been found in the NTS. Importantly, microinjection of each putative neuropeptide transmitter into the NTS produces a distinct set of physiological responses, while injection of antagonists to their receptors inhibits these physiological effects. Interestingly, the majority of these neuropeptides have had conflicting reports about their function when microinjected into the NTS, which supports the idea that they are involved in modulating rather than mediating visceral function. In the next section, evidence for the role of two novel neuropeptide transmitters expressed in vagal afferent neurons will be discussed as putative modulators of food intake.

6.1. Cocaine and amphetamine regulated transcript

Cocaine and amphetamine regulated transcript (CART) has been observed in approximately half of the neurons in the middle and caudal regions of the nodose ganglion [82–84]. Pinching experiments provide evidence that CART is transported along the vagus nerve [83]. Consistent with this idea, dense populations of CART fibers have been observed in the commissural and medial parts of the NTS [84,85], and these fibers were greatly reduced in response to supranodose vagotomy [84].

There is evidence that CART in vagal afferents plays a role in mediating the satiety induced by gastrointestinal signals. Retrograde labeling experiments demonstrate that CART positive neurons in the nodose ganglia innervate predominantly the duodenum, but also to a lesser extent the stomach [84]. Importantly CART extensively co-localizes with CCK1 receptors on vagal afferent neurons [83] and IP administration of exogenous CCK increases the number of CART positive cells in the nodose ganglia [82]. Postprandial release of CCK increases the number of neurons expressing CART and this is inhibited following administration of a CCK1 receptor antagonist [82]. Administration of CCK to cultured primary vagal afferent neurons dose-dependently induced CART synthesis and release [86]. In support of the idea that CART could mediate the satiating effects of the gastrointestinal

hormone CCK, IP administration of CART was found to prolong the satiating effects of exogenous CCK when administered together in rats after a short fast [86].

In addition to CCK, other gastrointestinal hormones have been implicated in regulating CART expression in nodose ganglia neurons. In cultured vagal afferent neurons leptin was found to potentiate CCK-induced neuronal expression of CART [87]. Leptin is released from chief cells of the stomach in response to nutrients, as well as from adipocytes in proportion to total body fat, however it remains unclear which site of leptin release is most important for the regulation of CART expression in vagal afferent neurons. The orexigenic gastrointestinal hormone ghrelin, released from A/X enteroendocrine cells of the stomach in the absence of the nutrients, was found to inhibit both the potentiating effects of leptin as well as the effects of CCK on CART expression in cultured vagal afferent neurons [82]. Orexin A, an orexigenic peptide expressed by a population of hypothalamic neurons with terminals that project to the NTS, has also been demonstrated to inhibit CART synthesis in response to CCK [71]. Thus, CART synthesis and release from vagal afferent neurons has been demonstrated to be regulated by hormones associated with feeding behavior.

There is evidence that both endogenous and exogenous CART inhibits food intake in the CNS. CART has been localized to brain regions associated with feeding behavior [85] and there is a large body of literature demonstrating intracerebroventricular (ICV) injection of CART dose-dependently reduces food intake [88–92]. Vrang et al. demonstrated that among the many different regions of the brain activated by ICV administration of CART, there was significant and prominent activation of NTS neurons [89]. Fourth ventricular injection of CART, to activate hindbrain neurons, reduces gastric emptying and inhibits gastric acid secretion [90,93], while stimulating colonic motility [94]. Hindbrain stimulation with exogenous CART also reduces the short-term intake of a liquid meal [95,96] independently of its effects on gastric emptying [96]. Interestingly, plugging the cerebral aqueduct between the third and fourth ventricles blocks the anorectic effect of ICV injection of CART [97], leading to the hypothesis that the anorectic properties of CART are solely mediated by the hindbrain. Microinjections of CART directly into the medial NTS was not found to reduce food intake in fasted rats [84]; however inhibiting endogenous CART, using an antibody against CART, has been demonstrated to prevent satiation in rats fed ad *libitum* [98] and preliminary data suggests that the endogenous CART requires fully functional vagal afferent neurons [99]. Crucially, silencing of CART in nodose ganglia neurons *in vivo* was found to abolish CCK-induced satiation [100], suggesting CART plays an important role in mediating the satiating effects of CCK.

To summarize, CART is expressed and released by vagal afferent neurons in response to a meal. CART is transported along vagal fibers, silencing CART expression in vagal afferent neurons prevents CCK-induced satiation, and endogenous CART in the NTS inhibits food intake. Taken together these data strongly suggest that CART is a novel neuro-peptide transmitter expressed by vagal afferent neurons that is involved in satiation. The mechanisms by which CART mediates its effects remain unclear, partially due to the fact that the CART receptor has not yet been identified. We know that CART can act presynaptically on vagal afferent fibers in the NTS [86] and there is some limited data suggesting that CART and glutamate can interact, at least in a spinal cord slice preparation.

In this preparation, CART was found to increase depolarization induced by NMDA-, but not AMPA, in substantia gelatinosa neurons [101]. Therefore it is possible to speculate that a similar mechanism may be involved in the NTS, whereby CART could depolarize postsynaptic NTS neurons to enable glutamate-induced NMDA receptor activation leading to satiation.

6.2. Melanin concentrating hormone

Melanin concentrating hormone (MCH) has recently been identified in mid- and caudal parts of the nodose ganglia [102]. MCH expression in vagal afferent neurons has been demonstrated to be regulated by the feeding state in rats; MCH abundance is high in the nodose ganglia of fasted rats, and is significantly reduced in response to a meal [102]. The vast majority of MCH-positive neurons in nodose ganglia co-localize with CCK1 receptor [102]. CCK was demonstrated to inhibit MCH expression in vagal afferent neurons; under fasting conditions, when MCH expression is high, CCK administration IP reduced the number of MCH-positive cells in the nodose ganglia. Conversely, postprandial reduction in MCH expression was reversed following administration of a CCK1 receptor antagonist [102]. In cultured vagal afferent neurons, CCK-induced downregulation of MCH was found to be prevented in cells transfected with a dominant negative mutant of the transcription factor cyclic-AMP response element binding protein (CREB) [82]. Exogenous administration of the gastrointestinal hormone ghrelin, released from the stomach in the absence of nutrients, attenuates postprandial-induced inhibition of MCH in nodose ganglia neurons [103]. Ghrelin was found to inhibit CCK-induced translocation of phosphoCREB to the nucleus, and therefore prevents active CREB from inhibiting MCH synthesis [82]. As a whole, these data suggest that MCH synthesis in vagal afferent neurons is upregulated by orexigenic gastrointestinal hormones and downregulated by the postprandial release of anorectic gastrointestinal hormones.

There is substantial evidence supporting the idea that MCH is involved in stimulating food intake [104–108]. Specifically, ICV injection of MCH increases food consumption [104,105] and ICV administration of MCH1 receptor antagonist reduces food intake [105]. MCH binds to a single G-protein-coupled MCH1 receptor that is widely expressed throughout the brain [109,110], including in the NTS. In the NTS of rats [7,111–113] and humans [114], MCH-positive fibers have been reported. Some MCH immunoreactive fibers are projections from hypothalamic MCH neurons; although the vast majority of the fibers are derived from another source [7]. The fact that NTS neurons activated by nutrient infusion into the stomach are targeted by MCH axons [7], suggests the possibility that a proportion of MCH fibers in the NTS are vagal afferents. This hypothesis is supported by unpublished data from our lab in which we found that nodosectomy significantly reduced MCH-positive fibers in the NTS.

In fasted animals, when endogenous MCH is highly expressed in vagal afferent neurons, fourth ventricular administration of MCH had no effect on food intake; however, it did reduce core body temperature without changing locomotor activity, suggesting a role in energy expenditure [7]. SNAP 94847, a MCH1 receptor antagonist, injected into the medial NTS inhibited food intake in fasted rats in a dose-dependent manner when compared to

saline [98]. These data suggest that endogenous MCH is released from vagal afferent neurons into the NTS under fasting conditions, resulting in a net increase in food intake. MCH administration to brain slices decreased the frequency, but not the amplitude, of spontaneous excitatory post-synaptic currents [7], suggesting that MCH may inhibit glutamate release by acting presynaptically on vagal afferent fibers. Interestingly, MCH and MCH1 receptor highly co-localize in the same vagal afferent neurons [102], and the same gastrointestinal signals control their gene expression [102,103]. These presynaptic events are likely to be more pronounced in response to prolonged food withdrawal since MCH1 receptor requires greater than 24 h fasting before becoming fully observed in vagal afferent neurons [115]. MCH1 receptors have also been identified on postsynaptic membranes [110] and could indicate a role for MCH in inhibiting glutamate activation of NMDA receptors by preventing depolarization of the postsynaptic neurons. Evidence in DMV brain slices that MCH decreases the amplitude of excitatory postsynaptic current induced by stimulation of vagal fibers [7] supports this concept.

7. CART and MCH as modulators

Importantly, the same neuron can express both CART and MCH [82]. CCK acts as a gatekeeper that depresses synthesis of the orexigenic peptide MCH while rapidly stimulating expression of the satiety peptide CART (see Fig. 3). The ability of CCK to switch the neurochemical phenotype of vagal afferent neurons is potentiated by leptin and inhibited by ghrelin, and therefore these neurons express proteins that reflect the nutritional status of the animal. The differential expression of these peptide transmitters with opposing function on feeding behavior provides evidence for a previously unsuspected plasticity to gut–brain signaling in response to nutrient availability.

It is important to note that CART and MCH synthesis in response to nutritional status is unlikely to have an immediate effect on the current meal. Although vagal afferent neurons can rapidly switch their neuro-chemical phenotype (see Fig. 3), the transport of these neuropeptides from the cell bodies to the afferent terminals in the NTS will be a rate-limiting step in translating protein expression to function. We have evidence that anterograde labeled vagal afferent fibers in the NTS of fasted rats are devoid of CART, but CART immunopositive fibers appear within 2 h of refeeding [99]. This suggests that CART can be synthesized and transported from cell bodies to terminals in the NTS within 2 h. Interestingly, biotinylated dextran injection into the nodose ganglia, to anterogradely label the fibers in the NTS, requires 5–10 days to reach the terminals. Therefore there is a discrepancy in the rate of transport between CART and the anterograde label.

Although not specifically studied in vagal afferent fibers, pulse-chase radiolabeling experiments in sensory neurons of the eye and spinal cord have identified five classes of axonal transport that can be broadly categorized into fast and slow transport [116]. Fast axonal transport, which includes anterograde transport of neuropeptides in vesicles along microtubules, has been demonstrated to occur at a rate of 200–400 mm per day (1–5 μm/s) in mammals. Slow axonal transport carries cytoplasmic and cytoskeletal elements at a rate of 0.2–1 mm/day. Proteins destined for fast axonal transport are required to be sorted and packaged in the Golgi [117], which may explain the difference in the rate of transport

between CART and anterograde labels (such as biotinylated dextran). Importantly, assuming neuropeptides are carried by fast axonal transport, this corroborates our finding that they can reach the NTS within 1–2 h. However this lag between peripheral stimulation and the availability of the neuropeptides at terminals in the NTS suggests that neuropeptides are most likely involved in mediating feeding behavior of subsequent meals (see Fig. 4). Energy restriction will result in vagal afferent neurons switching their neurochemical phenotype from CART expression to MCH expression. The longer the fast, the more MCH vesicles accumulate within vagal afferent fibers of the NTS. The first meal following a fast will result in the release of MCH alongside glutamate. MCH inhibition of glutamate will result in a reduction in satiation. Although further work is required to demonstrate the validity of the proposed hypothesis, it provides a rational for the observation that meal size and duration are increased following a fast. In response to the first meal, CART will be synthesized. As pools of MCH are depleted in the NTS, they will be replaced by CART, which will enable/potentiate the satiating effects of glutamate. Subtle changes in CART and MCH expression and release throughout the day provide some explanation for the variability in meal patters of chow fed rats (see Fig. 4).

8. The roles of transmitters in vagal afferent neurons

As discussed above, there is mounting evidence suggesting that CART and MCH play a role in the regulation of food intake, possibly by modulating glutamate-induced satiation. However visceral sensory neurons of the nodose ganglia express many different transmitters (see Table 1). These are expressed in small subpopulations of vagal afferent neurons that innervate different peripheral sites. Microinjection of the different transmitters expressed by vagal afferent neurons into the NTS results in a variety of physiological roles. Therefore, the plethora of peptides expressed by the different subpopulations of vagal afferent neurons may modulate the physiological effects of glutamate in response to peripheral stimuli. The idea that primary sensory neurons can exhibit pathway-specific neuropeptide expression has been previously reported. Gibbins et al. demonstrated that individual dorsal root ganglia neurons contain multiple neuropeptides and that different combinations of neuropeptides were expressed in neurons with different peripheral projection [118]. Interestingly McMahon et al. found that changing the visceral projection of sensory nerves changed the neuronal chemistry of the nerve [119], suggesting that the plasticity of afferent neurons is dependent on the peripheral milieu.

Isolating the exact physiological function of each transmitter expressed in vagal afferent neurons requires a multifaceted approach. Firstly, expression of the transmitter needs to be demonstrated in nodose ganglia. Secondly, retrograde tracing experiments must be performed to determine the visceral sites that these neurons innervate. Subsequently the physiological stimuli that regulate gene expression and synthesis of the transmitters must be identified, as well as the peripheral signals that mediate release of the transmitters from terminals in the NTS. Phenotypical changes produced by the release of the endogenous transmitter from vagal afferent fibers into the NTS should be ascertained. Finally, it should be determined if this is a direct or modulatory effect.

While immunohistochemistry and in *situ* hybridization studies have demonstrated the presence of protein and/or mRNA of an impressive array of transmitters, many of the other necessary studies have not yet been completed to fully understand the physiological role of these transmitters (see Table 1). Nevertheless, there is some evidence that substance P, nitric oxide, and GABA may all be involved in regulating cardiovascular function in response to peripheral signals from the heart. The tachykinin, neurokinin A, has been implicated in respiration in response to peripheral signals from the lungs. Along with the data presented above suggesting a role for CART and MCH in the control of food intake, there is evidence to support the concept of pathway-specific neuropeptide expression in vagal afferent neurons to regulate the varied physiological effects of glutamate.

9. Cardiovascular function

9.1. Substance P

Substance P has been observed in a scattered population of neurons in the rostral end of nodose ganglia [120,121]. Ligation of the vagus nerve causes accumulation of substance P only on the proximal side, suggesting that substance P is transported in vagal afferent fibers from its site of production in the nodose ganglia [120]. Densely labeled substance P terminals in the medial and commissural NTS are significantly reduced following capsaicin treatment [122]. In accordance with this, radioimmunoassay analysis of micropunches from the medial NTS of rats with ganglionectomy showed significantly reduced substance P levels [123].

The primary role of substance P in vagal afferents appears to be in modulating baroreflex control and baroreceptor-mediated blood pressure, though it may also be involved in respiration (see below). Retrogradely labeled studies from carotid sinus and aortic nerve, found a high degree of colocalization of retrogradely labeled nodose ganglia neurons with substance P [124]. Microdialysis experiments found that aortic nerve stimulation in the rat results in substance P release in the NTS [125]. Mechanical activation of baroreceptors in the thoracic aorta that produced baroreflex-dependent decreases in heart rate was accompanied by increases in substance P in the caudal NTS [126].

Three distinct tachykinin receptors exist, neurokinin receptor 1–3 (NK1–3). NK1, which has highest affinity for substance P, is strongly labeled in the medial NTS [127–129]. Vagal afferent fibers appear to be an important source of substance P for NTS neurons, since nodosectomy results in increased NK1 density in the NTS [130]. From extracellular recordings of cardioreceptive NTS neurons, NK1 antagonist CP-99,994 reduced the number of action potentials following electrical stimulation of the vagus nerve [131]. Microinjection of substance P antagonist into the NTS blocks the baseline or reflex effects of substance P [132,133], and ablation of NTS neurons that express NK1 prevents the gain of the baroreflex [134]. Microinjection of substance P into the NTS produced less clear results; in some instances substance P decreased resting blood pressure and heart rate [132,133,135,136], while in other instances substance P had no effect [133,137].

The inconclusive nature of the results produced by microinjection studies of substance P may point to a modulatory role of substance P release from vagal afferent fibers into the

NTS. This idea is supported by findings that substance P fibers in the NTS only account for approximately 10% of the axons in the nodose ganglia [120]. Furthermore, substance P appears to potentiate the effects of glutamate in the NTS. Subthreshold doses of substance P microinjected into the NTS of rats increased the depressor response of non-NMDA receptor agonist [138]. Blocking NMDA and non-NMDA receptors in the NTS prevented substance P-induced depressor and bradycardic response [139].

9.2. Nitric oxide

Nitric oxide (NO) is synthesized from L-arginine by nitric oxide synthase (NOS). Nodose ganglia neurons were observed to express the neuronal and endothelial isoforms of NOS (nNOS and eNOS) [140–142]. Fibers in the NTS also contain nNOS [143] and eNOS [142]. nNOS-immunoreactivity in vagal afferent fibers was significantly reduced by nodose ganglionectomy [143–145]. There is evidence that nitric oxide (NO) may participate in cardiovascular signaling from vagal afferent neurons to the NTS. A large proportion of nodose ganglia neurons retrogradely labeled from the aorta depressor nerve colocalize with nNOS [146]. Microinjection of S-nitrosocysteine, an NO donor, into the NTS decreases arterial pressure and heart rate [147,148]. Furthermore, L-NAME, a NOS inhibitor, evokes increases in arterial pressure and heart rate [149].

Extensive colocalization has been observed in fibers of the NTS between nNOS and vGluT2, a marker of glutamate uptake [150]. Importantly similar findings were reported in nodose ganglia neurons retrogradely traced from the aorta depressor nerve [146]. Pharmacological studies further support the idea that glutamate and NO may interact in the NTS to regulate cardiovascular function. L-NMMA, which inhibits the production of NO, significantly reduced depressor but not bradycardic responses elicited by L-glutamate [151]. A separate study found that both the depressor and bradycardic effects of glutamate or NMDA were blocked by prior administration of NO synthase inhibitors L-NMMA or L-NAME [152]. Responses to NMDA microinjected unilater-ally into the NTS of anesthetized rats were significantly attenuated in a dose-dependent manner by prior IP injection of the nNOS inhibitor 7-nitroindazole (7-NI) [153]. L-NAME microinjections into the NTS reduced both AMPA-evoked cardiovascular changes and NMDA-evoked falls in arterial pressure and heart rate [154].

9.3. Gamma-aminobutyric acid

GABA is expressed in a subset of vagal afferent fibers in the NTS, and the number of fibers are reduced in response to sectioning of the vagus nerve [23]. Approximately 20% of the neurons in the nodose ganglia were observed to express GABA [155]. GABA is also expressed by a number of NTS neurons and in fibers projecting from other regions of the brain, so it is difficult to delineate the source of GABA when trying to determine its functional role. However, microinjection of GABA agonists into the NTS increases blood pressure [156], suggesting that were GABA to be released from vagal afferent neurons it may regulate cardiovascular changes.

GABA$_A$ and GABA$_B$ binding sites are found in the NTS [157] and both receptor types appear to affect blood pressure control. GABA$_A$ antagonist enhances responses to aortic

depressor nerve stimulation [158,159]. Baclofen, a GABA$_B$ antagonist, injected into the NTS, increases blood pressure [160] and attenuates decreased blood pressure following electrical activation of the aortic depressor nerve [161]. Interestingly, evidence for cross-talk between GABA and glutamate has been demonstrated in hippocampal and cortical neurons [162].

10. Respiration

10.1. Neurokinin A

Neurokinin A (NKA) has been found in nodose ganglia neurons [163, 164]. Unilateral nodose ganglionectomy causes a significant decrease in the content of NKA fibers in the NTS of rats [165]. Increased levels of preprotachykinin-A mRNA, which encodes NKA were found in nodose ganglia following 24 h allergen challenge in guinea pigs [166]. NKA microinjection into the NTS reduced respiratory frequency and increased tidal volume [167,168]. Interestingly NK2 receptor, which has high affinity for neurokinin A, but not NK1, which has high affinity for substance P, were found to be involved in the excitatory modulation of inspiratory activity [169]. It is possible that NKA modulates the neuronal response to glutamate in the NTS since NKA has been shown to modulate NMDA and AMPA activation in neurons of the dorsal horn [170]; however this has not properly been tested in the NTS.

Substance P injection into the NTS has also been found to have a role in modulating respiration [171,172]. This is unsurprising seeing both NKA and substance P are products of the same precursor, and both NKA and substance P are colocalized in nodose ganglia neurons. Although NK2 has higher affinity for NKA, substance P is still able to activate NK2 receptors. Furthermore, guinea pigs exposed to second hand smoke for 5 weeks had increased coughing and bronchoconstriction [173], and substance P injected into the NTS has been shown to cause apnea in rats [172]. Therefore substance P may be involved in mediating the overlap in respiration and cardiovascular signaling [72].

11. Conclusion

Glutamate is an important neurotransmitter in visceral afferent neurons of the vagus nerve and is important in mediating a range of physiological functions within the NTS. Here, a model has been proposed to suggest a role for neuropeptide transmitters, expressed by nodose ganglia neurons and released in the NTS, in gating glutamate-induced activation of NTS neurons. There is strong evidence that vagal afferent neurons can integrate signals at least in response to nutritional status to change their neurochemical phenotype. This previously unappreciated plasticity can lead to changes in behavior. A more detailed analysis of the expression patterns of these transmitters and their function may provide novel insights into the pathophysiology of many diseases, especially metabolic syndrome. It is possible that these neuropeptide transmitters may also play important independent roles in coordinating the cross-talk between NTS neurons with different physiological roles to help integrate whole body physiology. This type of research can provide a link between environmental factors and diseases, such as the role of inhalants in obesity. The development of novel conditional knockout mice and viral-based approaches may help to

shed some light on the full range of effects of these neuromodulators in nodose ganglia neurons in health and disease.

Acknowledgments

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HIGHLIGHTS

- Role of glutamate in vagal afferent signaling

- Problems with glutamate as the only neurotransmitter in vagal afferent neurons

- Evidence for existence of neuropeptide transmitters in vagal afferent neurons

- Putative role of peptide transmitters in modulating glutamate signaling

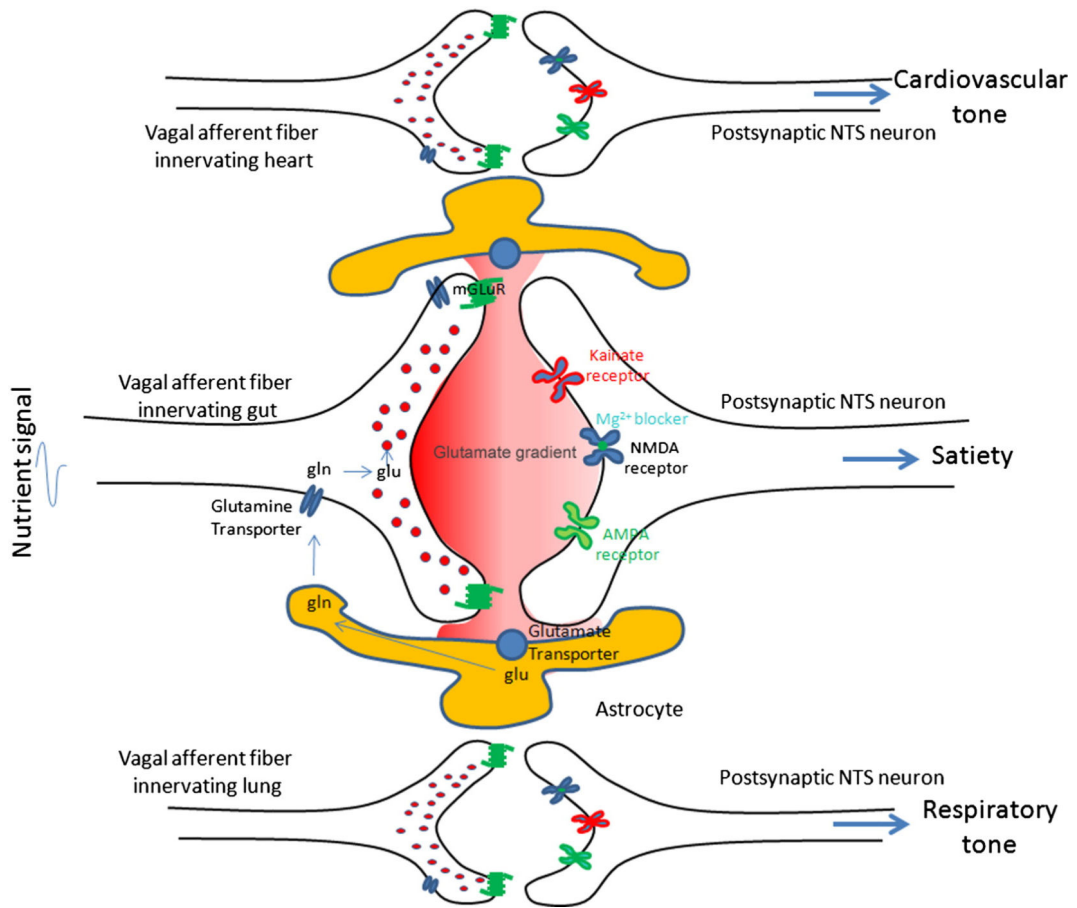- Plasticity provided by vagal peptide transmitters in regulating feeding behavior
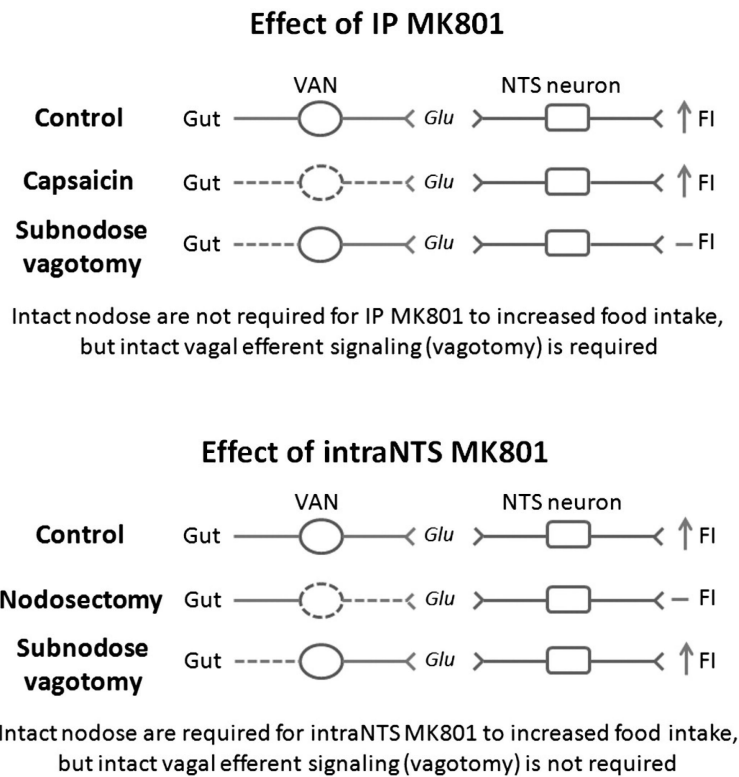
Fig. 1.
The role of glutamate in vagal afferent signaling. Vagal afferent fibers terminate on individual NTS neurons, enabling peripheral signals to mediate appropriate physiological responses (i.e. vagal afferent neurons innervating the heart controls cardiovascular tone; vagal afferent neurons innervating the gut controls satiation). Glutamate packaged in small vesicles in vagal afferent fibers is released into the synaptic cleft in the NTS in response to peripheral signals from visceral organs. The metabotropic glutamate receptor expressed on the presynaptic vagal afferent fibers regulates glutamate. Glutamate activates one or a combination of different ionotropic classes of receptors on postsynaptic NTS neurons mediating an appropriate physiological response. Astrocytes control temporal and spatial availability of glutamate, and minimize spillover, by regulating glutamate re-uptake. Glutamate transporters on astrocytes remove glutamate from the synaptic environment. Glutamate is converted to the inactive glutamine. Glutamine is released from astrocytes and transported into presynaptic neurons and repackaged into vesicles.

Effect of IP MK801

Intact nodose are not required for IP MK801 to increased food intake,
but intact vagal efferent signaling (vagotomy) is required

Effect of intraNTS MK801

Intact nodose are required for intraNTS MK801 to increased food intake,
but intact vagal efferent signaling (vagotomy) is not required

Fig. 2.
Systemic MK801 and intraNTS MK801 activate different pathways. Both intraperito-neal
and intraNTS administration of MK801 increase food intake. An intact vagus nerve is
required for systemic MK801 as demonstrated by the fact that vagotomy inhibits the
increases in food intake caused by IP MK801. However, vagal afferent neurons, as
demonstrated by capsaicin treatment, are not required for the orexigenic effects of systemic
MK801. Therefore vagal efferent neurons modulate the feeding effects of IP MK801.
Conversely, intraNTS MK801 was inhibited by nodosectomy, but not by sub-nodose
vagotomy. This suggests that vagal afferent neurons modulate the feeding effects of
intraNTS MK801. Thus, systemic MK801 and intraNTS MK801 administration activate
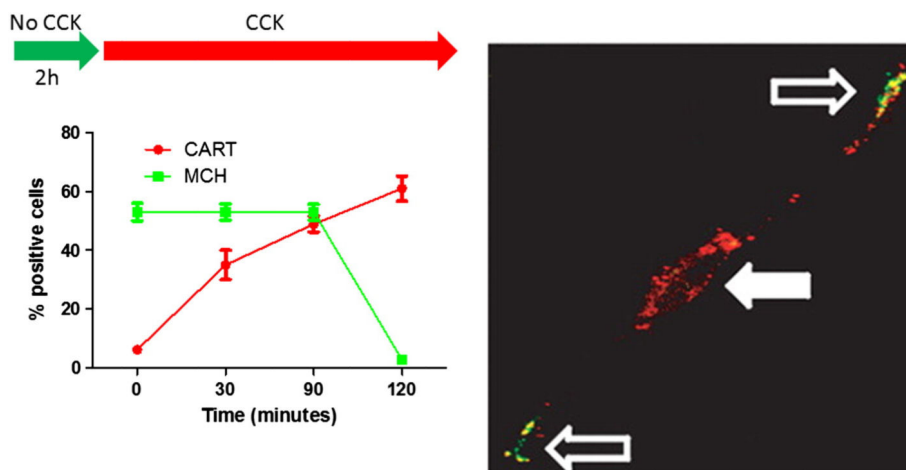different pathways.

Fig. 3.
The CART/MCH switch in vagal afferent neurons. Primary vagal afferent neurons cultured in the absence of CCK predominantly express MCH. Administration of CCK rapidly increases CART expression. Approximately 90 min after CCK administration, both CART and MCH can be observed in different pools of vesicles within the same neuron. CART is predominantly expressed in the perinuclear space in the cell body with some vesicles appearing in the processes, while MCH is exclusively observed in the terminals about to be released. Within 2 h of CCK administration, cultured vagal afferent neurons express predominantly CART. Thus the same vagal afferent neuron can switch between the expressions of different neuropeptide transmitters based on signals from the gut. Modified from [76].
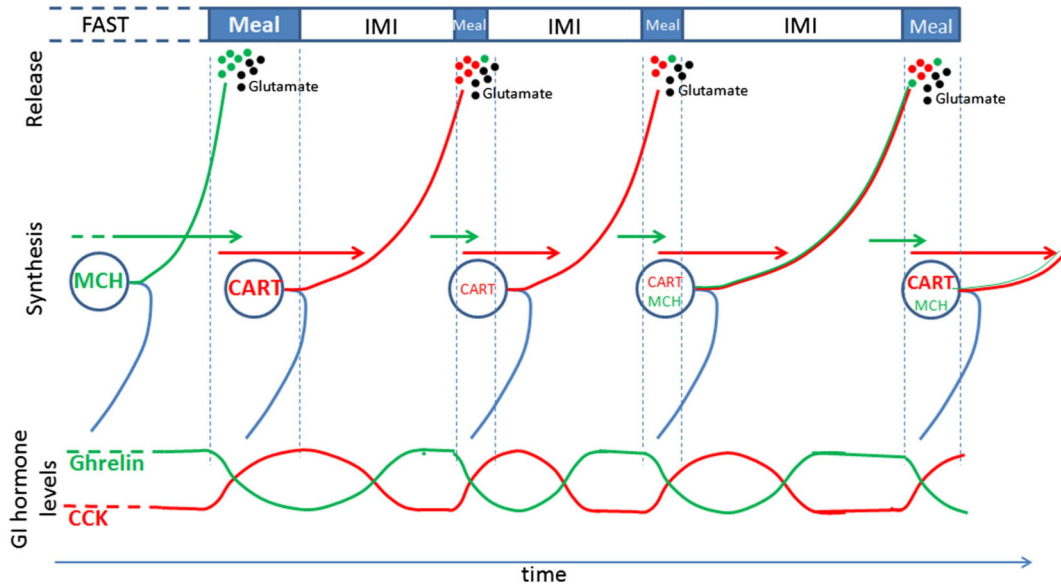
Fig. 4.
The role of CART and MCH in physiological feeding behavior. CART and MCH provide plasticity to the system enabling meal size and duration to change throughout the day. Importantly, CART and MCH synthesis and release in response to gastrointestinal signals will regulate feeding behavior of subsequent meals. MCH is synthesized in vagal afferent neurons and transported to the terminals during fasting periods; it is released into the NTS in response to a meal resulting in prolonged meal size and duration. CART is synthesized postprandially and transported during the intermeal interval; it is released during subsequent meals resulting in reduced meal size and duration. The switch in neurochemical phenotype of vagal afferent neurons enables tight control of glutamate-induced satiation. It remains unclear whether MCH is synthesized during intermeal intervals (IMI); however the release of both CART and MCH may explain the heterogeneity of meal size and duration over the course of the day in animals fed a diet with a set and unchanging nutrient composition.

Table 1

Amino acid based transmitters	Expression in nodose ganglia (NG) and vagal afferent fibers	Peripheral signaling	Functional role in the NTS	Intact vagal afferents required for function in NTS
Glutamate	•Glutamate immunoreactivity has been observed in NG and vagal afferent fibers [11-13] •Vesicular glutamate transporters (VGLUT) are expressed in vagal afferent fibers [15-16]. •Nodosectomy reduces glutamate uptake by 40% [14].	•VGLUT1 and VGLUT2 expressing VAN were retrogradely labeled to the trachea [173]. •VGLUT1 vagal afferent fibers retrogradely label the heart [16]. •VGLUT2 vagal afferent fibers retrogradely label the gut [16].	•Cardiovascular reflex [57], •Respiration [56] •Satiation (see this review), •Ingestion [4], •Motility [6], •Taste [59], •Inflammation [175] •Emesis [176]	Nodosectomy prevents endogenous glutamate induced satiation [52]
Gamma-aminobutyric acid (GABA)	GABA immunoreactivity has been observed in NG [23] and vagal afferent fibers in the NTS [155]	Unknown	•GABA injection into NTS increases blood pressure [156] •GABAA receptor antagonist increases aortic depressor nerve stimulation [158,159] •GABAB receptor antagonist increases blood pressure [160] and attenuates reduction in blood pressure following electrical activation fo the aortic depressor nerve [161]	Unknown
Nitric oxide (NO)	Nitric oxide synthase (NOS) expressed in nodose ganglia neurons [140,141]. and vagal afferent fibers in the NTS [142-145]	•3% of neuronal NOS (nNOS) expressing VAN were retrogradely labeled to the trachea [174], •A large proportion of nodose ganglia neurons retrogradely labeled from the aorta depressor nerve co- localize with nNOS [146]. •nNOS was expressed in about a third of all vagal afferent neurons projecting to the stomach [177] •eNOS is ubiquitously expressed in VAN [142]	•NO donor into the NTS decreases [147,148] while NOS inhibitor increases [149] arterial pressure and heart rate. •nNOS inhibitor (L-NAME), but noteNOS inhibitor (L-NIO) administered to gastroesophageal vagal afferent fibers enhanced mechanosensitivity [177]. The role of NO on glutamate release in the NTS was not tested therefore it remains unclear ifthis is a direct effect of NO or an inderect effect of modulating glutamate release.	Unknown
Acetylcholine	The enzyme choline acetyltransferase required for acetylcholine production is expressed in nodose ganglia neurons [178,179]	Unknown	Microinjection of nicotinic acetylcholine receptor agonist, nicotine, into the NTS produced a dose- dependent decrease in BP and HR[180]	Unknown
Dopamine	Immunoreactivity of both dopa decarboxylase [181] and tyrosine hydroxylase, required for dopamine synthesis, has been observed in NG [182,199]	Tyrosine-hydroxylase containing vagal afferent neurons project to the esophagus and stomach [183]	Microinjection ofdopamine into the NTS increased blood pressure and heart rate [184]	Unknown

Amino acid based transmitters	Expression in nodose ganglia (NG) and vagal afferent fibers	Peripheral signaling	Functional role in the NTS	Intact vagal afferents required for function in NTS
Serotonin	Serotonin (5-HT)-like immunoreactivity has been detected in cell bodies ofthe nodose ganglia [185] and vagal afferent fibers [186,187,197]	Unknown	•Blood pressure and heart rate are decreased by low doses of 5HT but increased by high doses of 5HT microinjected into the NTS [188]. •5HT containing vagal afferent fibers in the NTS come into closecontact with NTS neurons implicated in respiration [189]	Unknown
Peptide based transmitters	Expression in Nodose ganglia (NG) and vagal afferent fibers	Peripheral signaling	Functional role in the NTS	Intact vagal afferents required for function in NTS
Cocaine- and amphetamine-regulated transcript (CART)	Protein and mRNA for CART was identified in NG and in vagal afferent fibers in the NTS [82-84].	•CART is expressed in 17% of neurons that project to the stomach and 41% of vagal afferent neurons that project to the small intestine [83] •Protein expression was demonstrated to be differentially regulated in response to nutritional status: expressed postprandially in NG and vagal afferent fibers in the NTS [82]	•Inhibiting endogenous CARTin the NTS stimulates food intake [98]. •CARTacts as an autocrine regulator of gene expression in vagal afferentneurons [86].	Nodosectomy prevents endogenous CARTinduced satiation [98]
Melanin concentrating hormone (MCH)	Protein and mRNA for MCH was identified in NG and in vagal afferent fibers in the NTS [102,9,111-113].	Protein expression was demonstrated to be differentially regulated in response to nutritional status: expressed under fasting conditions in NG [82,102]	•Blocking endogenous MCH in the NTS inhibits food intake [98]. •MCH1 receptors are expressed by vagal afferent neurons in response to prolonged fasting, suggesting MCH may act as an autocrine regulator of vagal afferent signaling [7,99].	Nodosectomy prevents endogenous MCH induced food intake [98]
Substance P (SP)	•mRNA for preprotachykinin A (precursor to SP, NKA) found in nodose ganglia by in *situ* hybridization [190] and SP immunoreactivity was also observed in nodose ganglia [162,164,199] •Unilateral nodosectomy reduces SP immunoreactive vagal afferent fibers in the NTS [165].	•SP did not colocalize with nodose ganglia retrogradely labeled from the lung under basal conditions [191], but acute allergen exposure increases SP mRNA in vagal afferen neurons [166]. •Vagal afferent neurons retro-gradely labeled to the carotid sinus and aortic nerve colocalize extensively with SP [124] and activation of the aortic nerve increases SP expression in the NTS [125,126]	•Injection of SP into the NTS induced •central apnea [172] •mixed effects on blood pressure and heart rate, low doses decrease both[132,133,135,136,171], or having no effect on both [128,132] •Microinjection of SP antagonist into the NTS increases blood pressure and heart rate [133], •Ablation of NTS neurons that express NK1 receptor prevents the gain of the baroreflex [134].	Substance P dose dependently decreased the frequency but not amplitude of excitatory postsynaptic currents in NTS neurons receiving monosynaptic vagal afferent input from lungs and airways [212]. This suggests that SP released from vagal afferents into the NTS can modulate respiratory tone by inhibiting pre- synaptic

Amino acid based transmitters	Expression in nodose ganglia (NG) and vagal afferent fibers	Peripheral signaling	Functional role in the NTS	Intact vagal afferents required for function in NTS
			release of glutamate.	
Neurokinin A (NKA)	•mRNA for preprotachykinin A (precursor to SP, NKA) found in nodose ganglia by in *situ* hybridization [190] •Immunoreactive NKA was observed in nodose ganglia neurons [163] •Unilateral nodosectomy reduces NKA immunoreactive vagal afferent fibers in the NTS [165].	• Increased levels of preprotachykinin-A mRNA which encodes NKA were found in nodose ganglia following 24 h allergen challenge in guinea pigs [166].	•Microinjection of NKA into the NTS dose-dependently causes prompt, transient hypotension and brady-cardia [165]. •NKA microinjection into the NTS reduced respiratory frequency and increased tidal volume [167,168].	Unknown
Calcitonin gene-related peptide (CGRP)	CGRP immunoreactivity in NG and vagus nerve colocalizes with Sub stance P[192,163] and in vagal afferent fibers [193]	•CGRP expressed in NG retrograde-ly labeled to lung [191,194] •In the nodose ganlia the majority of the retrograde labeled nerver cell bodies from the esophagus stored CGRP [192]	Lesion of the area postrema/nucleus of the solitary tract (AP/NTS) attenuates CGRP-induced satiety [193]	Unknown
Cholecystokinin (CCK)	•CCK immunoreactivity was demonstrated in NG [118,160,195,196,198,199] •No preproCCK mRNA was identified in nodose ganglia under basal conditions [187] •There are reports that the antibody used mayalso bind CGRP [160].	•CCK was absent under basal condition but increased in response to nerve damage [200] •No change in response to food deprivation or high fat feeding [200].	Unknown	Unknown
Galanin	Galanin immunoreactivity and mRNA have been reported in nodose ganglia [201,202]	Retrogradely labeled galanin-positive vagal afferent neurons project to the stomach [203]	•Injection of galanin into the NTS: •stimulate feeding [204] •reduces blood pressure [205] •increases heart rate [205] •Galanin inhibits mechanosensitivity of vagal afferents, possibly by acting as an autocrine regulator [203].	Unknown
Neuropeptide Y (NPY)	In situ hybridization [190] and immunoreactivity [205] confirmed the presence of NPY in nodose ganglia	Unknown	Possible role in modulating blood pressure [206]	Unknown
Somatostatin	In situ hybridization [190] and immunoreactivity [163,199] confirmed the presence of somatostatin in nodose ganglia	Unknown	Inhibition of somatostatin receptor 5 in the NTS stimulates gastric emptying [207]	Unknown
Vasoactive inhibitory peptide (VIP)	Caudal distribution of VIP immuno- reactivity in vagal afferent neurons [163,199]	Vagal damage (ie. vagotomy) and inhibiting vagal transport significantly increased VIP	VIP injected in the Nucleus Tractus Solitarius- Dorsal Motor Nucleus complex reduced	Unknown

Amino acid based transmitters	Expression in nodose ganglia (NG) and vagal afferent fibers	Peripheral signaling	Functional role in the NTS	Intact vagal afferents required for function in NTS
		mRNA and immunoreactivity [208,209,210]	jejunal alanine and water absorption, and increased gastric acid release [211]	
Brain-derived neurotrophic factor (BDNF)	Abundantly expressed in adult vagal afferent neurons [213]	In cultured neurons. BDNF release is regulated by physiological patterns of baroreceptor activity [213]	Possible role in mediating baroafferent synapses [214]	Unknown