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Plasticity of vagal brainstem circuits in the control of gastric function

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Abstract

Background—Sensory information from the viscera, including the gastrointestinal (GI) tract, is transmitted through the afferent vagus via a glutamatergic synapse to neurons of the nucleus tractus solitarius (NTS), which integrate this sensory information to regulate autonomic functions and homeostasis. The integrated response is conveyed to, amongst other nuclei, the preganglionic neurons of the dorsal motor nucleus of the vagus (DMV) using mainly GABA, glutamate and catecholamines as neurotransmitters. Despite being modulated by almost all the neurotransmitters tested so far, the glutamatergic synapse between NTS and DMV does not appear to be tonically active in the control of gastric motility and tone. Conversely, tonic inhibitory GABAergic neurotransmission from the NTS to the DMV appears critical in setting gastric tone and motility, yet, under basal conditions, this synapse appears resistant to modulation.

Purpose—Here, we review the available evidence suggesting that vagal efferent output to the GI tract is regulated, perhaps even controlled, in an “on-demand” and efficient manner in response to ever-changing homeostatic conditions. The focus of this review is on the plasticity induced by variations in the levels of second messengers in the brainstem neurons that form vago-vagal reflex circuits. Emphasis is placed upon the modulation of GABAergic transmission to DMV neurons and the modulation of afferent input from the GI tract by neurohormones/neurotransmitters and macronutrients. Derangement of this “on-demand” organization of brainstem vagal circuits may be one of the factors underlying the pathophysiological changes observed in functional dyspepsia or hyperglycemic gastroparesis.

Keywords

vagus; brainstem; stomach; plasticity; receptor trafficking

Functional gastrointestinal motility disorders, which include functional dyspepsia (FD), are a common cause of visits to primary care and specialist physicians. Almost 45% of the US population report upper GI symptoms, and 20-30% of people complain annually of chronic or recurring symptoms of FD. Based on the Rome III panel recommendations, FD is defined as “the presence of symptoms thought to originate in the gastroduodenal region, in the absence of any organic, systemic or metabolic disease that is likely to explain the symptoms”¹. In many patients, FD include symptoms such as, amongst many others, impaired gastric emptying, reduced stomach compliance and weight loss¹⁻⁹. Although the pathophysiology of FD remains incompletely understood, several lines of evidence point toward impairment of vagal sensory-motor circuits connecting the gut to and from the central nervous system (CNS). The coordinated control of digestive and reflexive processes in the upper GI tract is under vagal control (reviewed in¹⁰). The involvement of the vagus

nerve is further emphasized by the observation that almost 50% of FD patients have disturbed efferent vagal functions and abnormal intragastric meal patterns with preferential accumulation of food in the distal stomach^{7, 11, 12} suggesting that impairment or defective accommodation of the proximal stomach may be involved..

Vago-vagal reflex control of the gastrointestinal tract

Extrinsic afferent visceral sensory information from the GI tract can be classified based upon either their pressure-response properties (mainly low-threshold, but also pain-related high-threshold fibers), the location of their receptive fields (i.e. mucosal, muscle, or serosal/mesenteric receptors), their stimulus-response properties (chemical or mechanosensitivity), their type of mechanoreceptors (in-parallel or in-series) or their region of origin along the GI tract (reviewed in¹³). Regardless of their function(s) or modalities, however, visceral sensory information is transmitted through the afferent vagus into the brainstem via a glutamatergic synapse at the level of the nucleus of the tractus solitarius (NTS)^{10, 14-17}. Second order neurons of the NTS have heterogeneous properties that contribute to the integration of synaptic inputs from the central terminals of vagal afferent neurons as well as from other higher CNS centers involved in the regulation of autonomic functions and homeostasis^{10, 18-21}. Information from these converging inputs is assimilated and integrated with metabolic and hormonal signals to shape the resulting output response. NTS neurons project to, among other areas, the adjacent dorsal motor nucleus of the vagus (DMV), which contains the preganglionic parasympathetic neurons that provide the vagal motor output to the GI tract. DMV neurons are, *a priori*, cholinergic and innervate postganglionic neurons located within the target organ of interest. Using an intact vagus-gastric myenteric plexus *in vitro* preparation, Schemann and Grundy²² demonstrated that stimulation of vagal preganglionic fibers, most probably originating from soma within the DMV, induces excitation in myenteric neurons of the guinea pig gastric corpus mediated primarily by activation of nicotinic receptors. These data indicate that acetylcholine is the principal neurotransmitter released from vagal efferent terminals and that preganglionic vagal fibers excite enteric neurons. In the stomach, postganglionic parasympathetic neurons form two distinct pathways: 1) an excitatory cholinergic pathway that increases gastric tone, motility and secretion via activation of muscarinic cholinergic receptors, and 2) an inhibitory non-adrenergic, non-cholinergic (NANC) pathway that inhibits gastric functions via release of predominantly nitric oxide (NO) or vasoactive intestinal polypeptide (VIP; reviewed in¹⁰). Gastric functions may be inhibited, therefore, either by activation of the NANC pathway or by inhibition of the tonic cholinergic pathway (Figure 1). Given the importance of the vagal reflexes in the control and integration of visceral functions, it is hardly surprising that malfunctions in vagal reflexes frequently result in, or are associated with, GI pathologies and digestive disorders including functional dyspepsia, gastroparesis, esophageal reflux, colitis, anorexia and bulimia nervosa, to name but a few^{4, 23-28}.

The vagal motor output to the GI tract arises from the neurons of the DMV. One of the remarkable, and fundamental, properties of gastric-projecting DMV motoneurons are their spontaneous, slow (1-2 action potentials.s⁻¹) pacemaker-like activity, the rate of which is modulated by synaptic inputs. Functional studies have determined that, despite the diverse biophysical and neurochemical properties of NTS neurons, they use primarily glutamatergic, noradrenergic and/or GABAergic inputs to innervate DMV neurons (reviewed in¹⁰). Microinjections of GABAergic antagonists into the dorsal vagal complex (DVC, i.e., NTS, DMV plus area postrema) increases gastric tone and motility dramatically, whereas microinjection of glutamatergic or catecholaminergic antagonists have little effect *per se* on gastric tone and motility^{10, 29, 30}. This observation argues in favor of a tonic GABAergic input from the NTS to DMV controlling the firing rate of gastric-projecting DMV neurons and, by consequence, the vagal motor output to the stomach.

One immediate implication is that the stomach, even at rest, is the recipient of vagal efferent outflow that is continuously sculpted by incoming signals (for example, sensory vagal, descending CNS and humoral inputs) modulating the activity of DMV neurons. An extraordinary degree of adaptive plasticity is required to ensure that vagally-regulated GI functions respond appropriately to a variety of intrinsic and extrinsic factors such as, for example, food, stress and even time of day. This is of particular importance since the inherent pacemaker activity of DMV neurons implies that even minor alterations in neuronal inputs can result in major changes of the vagal motor output and, by consequence, gastric motility and tone as well as response to vago-vagal reflexes.

Given the prominent role of GABAergic transmission within brainstem vagal circuits, it was rather surprising that studies from several laboratories failed to demonstrate modulation of GABAergic synaptic transmission to DMV neurons by neurotransmitters or modulators, bringing into question the physiological relevance, or benefit, of having such an important synapse that is apparently impervious to modulation.

cAMP-dependent plasticity at NTS-DMV synapses

Under basal conditions, GABAergic synaptic transmission from NTS to gastric-related DMV neurons appears unable to be modulated by, for example, μ -opioid peptides^{31, 32} or pancreatic polypeptides^{33, 34} because of the low resting levels of cAMP within NTS inhibitory nerve terminals. In a subset of synapses, however, it has been shown that raising cAMP levels, regardless of the mechanism, “uncovers” the ability of μ -opioid peptides^{31, 32} or pancreatic polypeptides receptors³⁴ to inhibit GABAergic synaptic transmission. It should be kept in mind, though, that evidence suggest that the same type of effect occurs at NTS-DMV synapses containing receptor systems that are also coupled negatively to adenylate cyclase, such as serotonin at 5HT_{1A} receptors³¹, oxytocin at OT-1 receptors³⁵ and norepinephrine at α_2 adrenoceptors³⁶.

Conversely, decreasing cAMP levels prevented the ability of adenylate cyclase activators to “allow” inhibition of GABAergic synaptic transmission. The rapid time-course of these actions (within 5 minutes) and the transient nature of the presynaptic inhibition (approximately 60 minutes) suggests that activation of the cAMP-PKA pathway within NTS GABAergic nerve terminals does not involve the *de novo* synthesis of new receptors, but rather induces the trafficking of pre-formed, internalized receptors to the nerve terminal membrane. Experiments using the Golgi-disrupting agent, Brefeldin-A, or low temperatures that stabilize the neuronal membrane, confirmed the involvement of a receptor trafficking pathway³² and further immunohistochemical studies demonstrated that activation of the cAMP-PKA pathway increases the co-localization of μ -opioid receptors with GABAergic nerve terminals, including terminals impinging upon gastric-related DMV neurons⁽³²⁾ (see Figure 2).

Thus, the levels of cAMP in a subset of GABAergic NTS terminals may determine the ability of neurotransmitters or neuromodulators to modulate inhibitory synaptic transmission to gastric-related DMV neurons. Under basal or resting conditions, cAMP levels in the terminals are low and receptors negatively coupled to adenylate cyclase are internalized rendering the synapse unavailable for modulation by neurotransmitters or modulators. Increasing cAMP levels or activating the cAMP-PKA pathway (increasing the “state of activation” of the nerve terminal), rapidly and transiently traffics these receptors to the neuronal membrane, allowing synaptic transmission to be modulated. Formulation of this hypothesis, however, immediately raised other questions: what keeps cAMP levels within NTS GABAergic nerve terminals low, and why should GABAergic, but not glutamatergic, NTS terminals be regulated in such a manner? Since vagally-mediated GI reflexes can occur

independently of higher CNS inputs, it would suggest that cAMP levels with GABAergic nerve terminals may perhaps be regulated by afferent (sensory) vagal inputs from the periphery.

Vagal afferent control of cAMP levels within GABAergic NTS terminals

Selective removal of vagal afferent inputs, either chemically³⁷ or surgically³⁸ was subsequently demonstrated to allow the inhibition of GABAergic synaptic transmission to gastric-related DMV neurons without the prior need to increase cAMP levels³⁹. Vagal deafferentation was also shown to increase cAMP levels within the brainstem and increase co-localization of μ -opioid receptors on GABAergic nerve terminals³⁹ (See Figure 3).

The levels of cAMP within subsets of brainstem nerve terminals may not only be important *modulators* of neurotransmitter release, but under certain circumstances, they may actually *control* the release of neurotransmitter. cAMP can facilitate synaptic transmission through actions at multiple sites, ranging from regulation of the availability of intracellular calcium, to the sequence of events leading to exocytosis⁴⁰⁻⁴³. The stimulus-dependent insertion of proteins and receptors into the plasma membrane occurs commonly in many systems, including the CNS⁴⁴⁻⁴⁷. In fact, previously silent synapses have been shown to release neurotransmitters spontaneously following activation of the cAMP/PKA pathway⁴⁰. An increase in the intracellular levels of cAMP is also likely to result in an increased sensitivity of the synapse to activation of receptors negatively coupled to adenylate cyclase, such as μ -opioid, 5-HT_{1A}, pancreatic polypeptide Y1 and Y2 as well as oxytocin OT1 receptors.

As mentioned above, vagal afferent fibers transmit sensory information into the brainstem via a glutamatergic synapse^{10, 14-17}. Activation of ionotropic glutamate receptors (i.e., NMDA and non-NMDA receptors) is crucial in the transmission of visceral sensory information but several metabotropic glutamate receptors (mGluR) have also been identified within the DVC⁴⁸. The mGluR present in the DVC include group II and group III mGluR that are located predominately at presynaptic sites and are negatively coupled to adenylate cyclase. Their metabotropic nature implies that glutamate can exert long-lasting effects on sensory integration and synaptic transmission^{15, 48-54} and, by consequence, could play important roles in the regulation of cAMP levels and modulation of synaptic transmission in GABAergic NTS terminals. In fact, ongoing activation of group II, but not group III, mGluR appears critical in regulating cAMP levels within GABAergic NTS terminals⁵⁵. Antagonism of group II mGluR elevates cAMP levels sufficient to allow translocation of μ -opioid receptors to the terminal membrane where their activation inhibits synaptic transmission to gastric-related DMV neurons. Removal of vagal afferent inputs (which provide a tonic glutamatergic input) relieves the ongoing activation of these group II mGluR receptors, hence eliminates the need to exogenously increase cAMP levels in order to modulate inhibitory synaptic transmission. Such modulation appears, at least initially, rather cumbersome and awkward. On further reflection, however, this may actually be an extremely convenient and metabolically “cheap” means by which visceral afferent inputs can control the efferent motor response in an ‘on-demand’ and responsive manner^{55, 56}. Vagal afferent inputs already utilize glutamate as their principal neurotransmitter onto NTS neurons. By using the same neurotransmitter to activate metabotropic, rather than ionotropic, receptors, vagal afferent inputs can regulate the tonic GABAergic input onto DMV neurons hence set the “tone” of vagal efferent motor output. Thus, visceral inputs themselves are easily able to “gate” the output efferent response as required by homeostatic demands.

Surprisingly, given their apparent insensitivity to modulation by cAMP, glutamatergic NTS terminals also display functional group II and group III mGluR. The principal difference

between NTS glutamatergic and GABAergic terminals appears, therefore, to be the lack of monosynaptic input from vagal afferent fibers. Because they do not receive this direct monosynaptic input from vagal afferents, neither the group II nor the group III mGluR expressed on glutamatergic NTS terminals are activated tonically. By consequence, cAMP levels within glutamatergic NTS nerve terminals is not suppressed and excitatory synaptic transmission to gastric-related DMV neurons is open to modulation, even in the basal state. This implies that anatomical organization of vagal afferent inputs to glutamatergic and GABAergic NTS-DMV synapses are fundamentally different and suggests that vagal brainstem neurocircuitry is anatomically quite distinct (see Figure 4).

Plasticity of vagal afferent transmission

Plasticity within GI vagal brainstem circuits may not be restricted to the NTS-DMV GABAergic synapse, however. Recent evidence suggests that the first synapse in vago-vagal reflexes, between vagal afferent terminals and NTS neurons, may also exhibit plasticity and be regulated by visceral inputs. Several feeding-related neurohormones/neuromodulators, including CCK^{57, 58}, serotonin⁵⁹, and melanocortins⁶⁰ can modulate glutamatergic synaptic transmission from vagal afferent terminals onto NTS neurons. Recent evidence suggests that even an elevation or reduction in extracellular glucose levels may increase or decrease glutamatergic transmission, respectively⁶¹ via an alteration in either the number and/or the function of presynaptic 5-HT₃ receptors⁶². While glucose levels within the CNS are undoubtedly lower than those found peripherally they do appear to alter in concert with blood glucose levels, albeit within much a narrower range^{63 64}. Furthermore, because the DVC is essentially a circumventricular organ with large areas being either entirely outside the blood brain barrier (e.g., the area postrema) or having a 'leaky' blood brain barrier because of the presence of fenestrated capillaries (e.g. NTS and DMV)^{65, 66}, neurons within the DVC are more likely to be accessible to circulating factors, including glucose⁶⁷ and feeding-related neurohormones³⁸.

Plasticity of vagal afferent neurons

Plasticity within vagal afferent neurons themselves have been the subject of several recent excellent reviews^{68, 69}, which are recommended strongly to readers. In brief, the expression of G-protein coupled receptors on vagal afferent neurons, hence their response to neuroendocrine and gastrointestinal signals, is subject to a remarkable degree of on-going plasticity, particularly as it relates to the recognition or 'perception' of recent food intake. For example, in addition to modulating synaptic transmission from vagal afferent central terminals³⁸ and permitting the modulation of GABAergic transmission at NTS-DMV synapses (see above), CCK appears to function as a "gatekeeper" that regulates sensory afferent signaling from the periphery by regulating the balance of orexogenic *versus* anorexogenic responses.

Following food ingestion, CCK is released from enteroendocrine I cells and activates vagal afferent neurons via actions at CCK₁ receptors, resulting, ultimately, in gastric relaxation and early satiety⁷⁰⁻⁷². In addition to its ability to modulate vagal afferent neuronal activity directly, however, CCK also decreases the expression of 'anorexigenic' G-protein coupled receptors such as cannabinoid (CB1), melanin concentrating hormone (MCH-1) while increasing the expression of 'satiety' G-protein-coupled receptors such as cocaine and amphetamine-regulated transcript (CART) and pancreatic polypeptide Y2 receptors^{68, 69}. CCK 'switches' the phenotype of vagal afferent neurons to signal a 'fed' response. As CCK levels decrease, following fasting for example, the expression of CB1 and MCH-1 receptors increases while the expression of Y2 and CART decreases, switching the phenotype of vagal afferent neurons to that of a 'fasted' response. This implies that vagal afferent neurons are

remarkably labile with the ability to alter their phenotype rapidly as homeostatic circumstances dictate.

Functional Relevance & Functional Dyspepsia

Although the relative contribution varies highly between different subgroups of patients, it has long been recognized that the symptoms of FD are often aggravated by food ingestion and/or psychosocial factors, such as stress. Interestingly, both food ingestion and stress release neurotransmitters, neuromodulators and neurohumoral agents, such as CCK, GLP-1 and CRF, that appear capable of modulating vago-vagal reflexes at multiple sites, from altering the phenotype of vagal afferent neurons, to regulating the release of glutamate from the central terminals of vagal afferents, to regulating the strength of GABAergic inputs onto gastric projecting vagal efferent neurons. It is well accepted that stress-related alterations of gastric motility are among the main pathophysiological mechanisms involved in the genesis of dyspeptic symptoms, and a majority of FD patients associate stressful life events with initiation or exacerbation of their symptoms^{73, 74}.

Based on data from several laboratories, we suggest the following working hypothesis: Vagal afferent terminals release glutamate on gastric related NTS neurons. During periods of “rest” or minimal visceral feed-back, tonic release or spillage of glutamate from vagal terminals is insufficient to evoke “full-scale” vago-vagal reflexes but, because of the greater affinity of glutamate to mGluR compared to iGluR, these low levels of glutamate released at a subset of NTS-DMV GABAergic synapses are sufficient to activate mGluRs present at the NTS-DMV synapse. Activation of these mGluR tonically inhibits vagal circuits by suppressing cAMP levels; receptors negatively coupled with adenylate cyclase are internalized at the GABAergic terminals and, thus, are unavailable for modulation. This situation leaves the vagal output controlling the stomach in an idle state, although sufficient to perform interdigestive tasks. As a result, a metabolically “inexpensive” neurotransmitter, such as glutamate, exerts profound, long-lasting dampening effects over the activity of vago-vagal circuits.

In response to food ingestion, or stressful events, however, circulating hormones or local neurotransmitters activate vago-vagal reflexes but additionally modulate the sensitivity of vagal neurocircuits at multiple sites. As a result, the vagal efferent outflow to the viscera is more capable of undergoing the necessary plastic adaptive changes required to prepare the stomach for appropriate ingestive and digestive processes. In FD patients, or indeed in any situation where there release of gastrointestinal neurohormones may be dysregulated or compromised, these adaptive changes are deranged and the disrupted sensitivity of the sensory-motor vagal circuitry results in altered gastric motility. In fact, one may consider that in FD, these circuits are in a constant “state of activation” due to the enhanced release (or coupling) of stress or meal related hormones. By consequence, the plasticity and adaptability induced normally by an otherwise harmless situation, such as meal ingestion, are now processed inappropriately and result in disrupted gastric functions.

Conclusions & Future Directions

There has been a recent, and growing, awareness that vagally-mediated reflexes exhibit a high degree of modulation and plasticity and cannot be considered static relay networks producing stereotypical output responses to visceral signaling. Within the GI system, visceral information either in the form of vagal afferent activity itself, as GI-related neurohormones released in response to meal ingestion or as macronutrients themselves, are able to alter the parasympathetic motor output via modulation of the activity of brainstem neurotransmission. The timing of these responses (minutes-hours) appears well suited to

respond to the homeostatic demands of the gastrointestinal tract. Clear differences are immediately apparent, however, when compared to other autonomic brainstem reflexes. Modulation and plasticity with baroreflex brainstem circuits are observed only after prolonged periods (1-5 weeks) of hypertension, for example, ⁷⁵⁻⁷⁸ suggesting that brainstem vagal circuits are organized into anatomically and functionally specific circuits.

There are a great many questions that still remain to be answered regarding the plasticity of vagal brainstem circuits. Clearly, malfunctioning of vago-vagal reflexes controlling the GI tract can result in serious and long-term health consequences but the precise mechanism(s) responsible for the breakdowns in autonomic and homeostatic control remain to be discovered. Although, tonic vagal afferent input is crucial in setting the output “tone”, little is known about the consequences of long-term increased or decreased vagal afferent signaling. Similarly, little is known about the effects that long-term alterations in the release of GI neurohormones may exert upon vagally mediated reflexes. Likewise, while the plasticity in vagal neurocircuitry described herein are all readily reversible, under what circumstances would more permanent re-modeling of brainstem vagal neurocircuits, such as those that may be responsible for more chronic GI disturbances such as FD or persistent reflux, be induced? Is there a critical embryonic or neonatal period during which vago-vagal circuits are hard-wired? What role do disturbances during development play in “setting” the circuits and the “gain” of plasticity? Is plasticity within vago-vagal brainstem circuits a generalized phenomenon across all second messenger systems? Clearly, a concerted effort should be made between basic and clinical scientists utilizing different experimental approaches to understand the modulation of vagal reflexes in the regulation and control of autonomic homeostatic functions in both normal health as well as pathophysiological conditions.

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Figure 1. Schematic diagram illustrating vago-vagal reflex control of the stomach

The left-hand side of the diagram illustrates a schematic diagram of vagally-mediated gastric reflexes. The right-hand side illustrates a photomicrograph of a rat brainstem taken at an intermediate level of the dorsal vagal complex following prior application of the neuronal tracer DiI to the nodose ganglion. This neuronal tracer travels in both the retrograde and anterograde direction, labeling vagal efferent motoneurons within the dorsal motor nucleus of the vagus (DMV) as well as vagal afferent fibers in the tractus solitarius (TS) and their terminals within the nucleus of the tractus solitarius (NTS).

Sensory information from the GI tract is relayed centrally via the afferent vagus nerve, the cell bodies of which lie in the paired nodose ganglia. The afferent signal enters the brainstem via the tractus solitarius (TS), terminating with the nucleus of the tractus solitarius (NTS), utilizing predominantly glutamate as a neurotransmitter. NTS neurons integrate the visceral afferent signals with inputs from other brainstem and higher CNS nuclei, transmitting the combined response to, among other areas, the adjacent dorsal motor nucleus of the vagus (DMV), which contains the preganglionic parasympathetic motoneurons that provide the principal motor output to the stomach.

DMV neurons are, *a priori*, cholinergic, and activate nicotinic cholinergic receptors on postganglionic neurons within the target organ of interest, in this case, the stomach. Postganglionic neurons form two distinct pathways; a cholinergic excitatory pathway that, when activated, increases gastric motility and tone via activation of muscarinic cholinergic receptors on gastric smooth muscle, or a non-adrenergic, non-cholinergic pathway that, when activated, decreases gastric tone and motility via release of predominantly NO and VIP. Thus, inhibition of gastric functions can occur via either withdrawal of tonic cholinergic activity, or via activation of the NANC pathway.

Figure 2. Schematic diagram illustrating the cAMP-dependent trafficking of Gi/o coupled receptors on GABAergic NTS terminals

GABAergic NTS neurons provide a tonic inhibitory input onto vagal efferent DMV motoneurons. Glutamate released from vagal afferent (monosynaptic) terminals activates mGluR present on GABAergic terminals, decreasing cAMP levels within the nerve terminal (A). This, in turn, results in internalization of Gi/o coupled receptors removing their ability to modulate inhibitory synaptic transmission between NTS and DMV. Exposure to neurohormones such as the satiety peptides CCK and GLP-1 or the stress neurotransmitter CRF activates adenylate cyclase and increases cAMP levels within the nerve terminal, overcoming the effects of mGluR activation (B). This results in trafficking of Gi/o coupled receptors to the nerve terminal, allowing their activation to modulate inhibitory synaptic transmission to DMV neurons, the output of which is then significantly increased.

Figure 3. Deafferentation increases co-localization of oxytocin receptors on GABAergic nerve terminals within the brainstem and allows modulation of inhibitory synaptic transmission between the NTS and DMV

A: In a coronal slice (40 μ m thick) at a rostral level containing the DVC, the intact side of the brainstem (right) receives a dense vagal afferent innervation highlighted by rhodamine dextran-labeled vagal afferent terminals present within the tractus solitarius (TS) or NTS (area c, higher magnification C below). Conversely, the deafferented side of the brainstem (left; area b, higher magnification B below) does not contain rhodamine-labeled fibers. Note that the preganglionic motoneurons of the DMV are labeled on both sides of the brainstem, confirming the selectivity of the surgical deafferentation.

D: Brainstem slices (40 μ m) containing the DMV were processed for immunohistochemical localization of the oxytocin-1 receptor (red) and GAD (green) used as a marker for GABAergic nerve terminals. Following deafferentation (upper micrograph), the number of oxytocin-1 receptors (red) co-localizing with GABAergic nerve terminals (green) is increased (yellow; arrows), particularly apposing a Neurobiotin-filled DMV neurons (blue). In contrast, in a vagally-intact brainstem (lower micrograph) few, if any, points of co-localization can be observed.

E: Representative whole cell patch clamp recordings from DMV neurons in control (left) and deafferented (right) brainstems showing that in vagally-intact brainstems, oxytocin had no effect upon the amplitude of evoked GABAergic currents (left) whereas oxytocin decreased the amplitude of evoked GABAergic currents in DMV neurons recorded from deafferented brainstems.

Figure 4. Proposed schematic representation of neurohormone-mediated regulation of vago-vagal reflexes

Schematic diagram illustrating the various sites that neurohormones/neuromodulators can act to modulate or regulate vagally-mediated gastric reflexes. Neurohormones can activate vagal afferent neurons but can also regulate neuronal phenotype, hence adapt the responses of the GI tract to the “on-demand” needs to maintain proper homeostatic functions.

Neurohormones and macronutrients such as glucose may also regulate the ability of vagal afferent central terminals to release glutamate, hence modulate the ability of visceral signaling to be relayed centrally. Finally, neurohormones and neuromodulators may affect inhibitory synaptic transmission to gastric-projecting DMV neurons by controlling cAMP levels within GABAergic NTS terminals. It is readily apparent, therefore, that the integrated output vagal efferent response relayed back to the gastrointestinal tract is not a stereotypical unmodulated response but, rather, is exquisitely open to modulation.