Published in final edited form as:

Physiol Behav. 2011 November 30; 105(1): 94–99. doi:10.1016/j.physbeh.2011.02.042.

A Tale of Two Endings: Modulation of Satiation by NMDA Receptors On or Near Central and Peripheral Vagal Afferent Terminals

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Abstract

Glutamate is the neurotransmitter responsible for fast excitatory transmission from vagal afferents to second order neurons in the NTS. Antagonism of NMDA-type glutamate receptors in the NTS increases food intake and attenuates reduction of food intake by vagally mediated satiation signals, such as cholecystokinin. Although, the cellular location(s) of NMDA receptors that participate in satiation is uncertain, recent findings suggest that attenuation of satiation by NMDA receptor antagonists is due, at least in part, to their action on primary vagal afferents themselves. While evidence is accumulating that NMDA receptors located on vagal afferent endings in the hindbrain are involved in control of food intake, there also is preliminary evidence that peripheral NMDA receptors also may influence vagal control of food intake. Hence, NMDA receptor expression on central and perhaps peripheral vagal afferent endings could provide a parsimonious mechanism for modulation of satiation signals by endogenously released glutamate.

Keywords

vagus; control of food intake; gastrointestinal; satiety

In rats, the right and left nodose ganglia contain cell bodies of roughly 17,000 pseudounipolar neurons -- the primary vagal afferents [1]. The distal axon endings of these neurons innervate internal organs, while the proximal endings synapse in the nucleus of the solitary tract of the hindbrain (NTS), functionally tying the viscera to the brain. An estimated 70% of vagal afferents innervate the abdominal viscera, especially the stomach and intestines (For review see [2]). One of the functions of abdominal vagal afferents is to participate in the control of food intake by responding to gastrointestinal stimuli [3]. Although information has accumulated steadily regarding the sensitivity of vagal afferents to specific gastrointestinal stimuli, much less is known about the mechanisms by which gastrointestinal signals are transmitted to higher order neurons or modulated by transmitter receptors on central and/or peripheral vagal afferent endings.

Vagal afferents synthesize and apparently release a variety of neuroactive substances, including purines, amines, amino acids and peptides. Nevertheless, several types of evidence indicate that the excitatory amino acid anion, glutamate, is a major neurotransmitter for

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vagal afferent neurons. For example, vagal afferent terminals express immunoreactivity for vesicular glutamate transporters [4-8], which is indicative of glutamatergic neurotransmission. In addition, electrophysiological experiments reveal that glutamate is released from central vagal afferent terminals during electrical or chemical stimulation [9-11] triggering glutamate-mediated postsynaptic currents in NTS neurons. Finally, glutamate receptor antagonists applied to the NTS are reported to attenuate a number of physiological responses evoked by vagal afferent stimulation [12-16]. Clearly, glutamate and its cognate receptors play a pivotal role in vagal sensory function.

Neuronal responses to glutamate are mediated by three families of ionotropic glutamate receptors, (AMPA-, kainate- and NMDA-type) [17-20] and by several groups of G-protein coupled, metabotropic receptors [21]. Most electrophysiological observations indicate that vagal afferent terminals excite NTS postsynaptic neurons by activating AMPA-/kainate-type receptors, and to a lesser extent by actions at NMDA-type receptors. Although the traditional view of ionotropic glutamate receptors, including NMDA receptors, is that they are located on postsynaptic cell bodies and dendrites, NMDA receptor subunit immunoreactivity has been localized to axon terminals at a variety of sites in the brain [22, 23], including on vagal afferent terminals in the NTS [24]. Furthermore, electrophysiological data indicate that NMDA receptors located on axon terminals modulate transmitter release in many areas of the central nervous system, including the visual cortex [25], entorhinal cortex, [26], and primary somatosensory afferent terminals in the spinal dorsal horn [27-29]. Thus, it now appears that NMDA-type glutamate receptors, located on axon terminals, play an important role in determining the amount and types of chemical transmitters released onto postsynaptic receptors.

Intriguingly, mRNA coding for NMDA receptor subunit has been detected in the nodose ganglia by in situ hybridization (ISH) [30] and polymerase chain reaction (PCR) [31]. In addition, reports by Czaja et al. (Fig. 1) indicate that NMDA NR1 subunit immunoreactivity is present in more than 90% of vagal afferent cell bodies in the nodose ganglia [1], while expression of NMDA NR2 subtypes reveals that vagal afferents comprise several apparently distinct phenotypes, with regard to the composition of their NMDA receptors [32]. Finally, using ultrastructural methods, others have demonstrated the presence NMDA NR1 immunoreactivity on vagal afferent terminals in the NTS [24].

Because of the evidence that NMDA receptors are expressed by vagal afferents, and are localized both presynaptically on central vagal afferent terminals and postsynaptically on some hindbrain neurons innervated by vagal afferents, we postulated that NMDA receptors might participate in control of food intake by the vagus. Accordingly, in 1997 we published our first report demonstrating that rats increased their food intake following intraperitoneal injection of MK-801, a non-competitive, open channel blocker of NMDA receptor ion channels [33]. The results of these initial experiments revealed several interesting aspects of food intake associated with NMDA receptor blockade. First, MK-801 increased intake of either solid rodent diet or 15% sucrose solution (Fig. 2) following overnight food deprivation. The antagonist did not increase water intake following water deprivation. These results suggested that the effects of MK-801 were selective for food intake. In addition, we observed that when rats were not food deprived, MK-801 failed to trigger initiation of food intake. However, if MK-801 was administered to non-fasted rats that then were offered cookies, a highly palatable preferred food, intake was dramatically enhanced. These results suggested to us that the net effect of systemic NMDA ion channel blockade was to delay termination of food intake once it started. This hypothesis was subsequently supported by results from our lab, which demonstrated that if fasted rats were allowed to eat for 10 to 15 min prior to injection of MK-801 (Fig. 3), the antagonist did not further increase intake [34]. In order to be effective the drug had to be on board before or soon after feeding commenced.

It was ineffective once the process of satiation was complete. Subsequently, additional results were reported by Jahnig and Houtp [35], who examined the effect of MK-801 on the first and second nighttime meals in rats. They found that IP injection of MK-801 just prior to the start of nighttime feeding increased meal duration, but did not shorten the interval between meals, supporting the hypothesis that MK-801 interfered with meal termination or the process of satiation.

Lesions of the caudal NTS abolish increased food intake in response to IP MK-801 injections [36]. This finding suggested that the dorsal hindbrain was a site that is necessary in order to MK-801 to increase food intake but does not actually indicate the relevant receptors are located there. However, we also found that MK-801 injected via the fourth ventricle or directly into the NTS increased food intake in a manner similar to systemic MK-801 injection, but at much lower doses [37, 38]. In addition, Covasa and coworkers subsequently demonstrated that a variety of competitive NMDA receptor antagonists also increased food intake when injected via the fourth ventricle or into the NTS [39-41]. These results support the conclusion that NMDA receptors in the dorsal hindbrain participate in control of food intake. In addition, the fact that chemically diverse, competitive NMDA receptor antagonists, injected into the hindbrain, produce effects on food intake that are comparable to those produced by MK-801 suggest that these effect are indeed due to NMDA receptor-specific antagonism, as opposed to a chemical effect that is unrelated to NMDA receptor function.

The fact that the dorsal hindbrain, where vagal afferents terminate, is a site where NMDA receptors participate in control of food intake prompts the question of whether NMDA receptor activation is necessary for the processing vagally mediated satiation signals. In this regard it is very well documented that the gut peptide, cholecystokinin (CCK) reduces food intake by activating vagal afferents innervating the gastrointestinal tract [42], and we were encouraged by a prior report that MK-801 attenuated reduction of liquid diet consumption by rats fed through a chronically implanted cheek fistula [43]. In our own initial test for NMDA receptor participation in CCK-induced reduction of food intake we found that IP injection of MK-801 attenuated reduction of food intake by IP CCK-8 in freely feeding rats [44]. More recently, Guard et al. [40] have reported that systemic injection of the competitive NMDA antagonist D-CPPene not only attenuates CCK-induced reduction of food intake, but also attenuates CCK-induced increases of NTS Fos immunoreactivity, indicating that somewhere between the gut and the NTS NMDA receptors are necessary for CCK's vagally mediated effect on feeding and neuronal activation. Finally in experiments completed very recently we found that injection of MK-801 directly into the NTS prevented reduction of food intake by IP CCK (Fig. 4). Moreover, when D-CPPene was injected into the fourth ventricle we were able to prevent CCK-induced reduction of food intake and attenuate CCK-induced increase in NTS Fos expression [45]. These results strongly support the hypothesis that activation of NMDA receptors within the hindbrain itself is required in order for CCK to reduce food intake.

As noted earlier NMDA receptors are expressed by vagal afferents as well as by neurons in the NTS. Consequently, it is possible that NMDA receptor antagonists affect food intake via actions at postsynaptic NMDA receptors on NTS neurons or by occupying presynaptic NMDA receptors located on vagal afferent terminals. Currently there is little direct evidence to support one site of action over the other. Nevertheless, results from the following experiment are consistent with the hypothesis that presynaptic NMDA receptors, located on central vagal afferent endings, are necessary for enhancement of food intake by NMDA antagonists. This experiment is summarized in Fig. 5. Briefly, the rationale, execution and interpretation of our experiment were as follows. Rats in which the vagi have been cut below the diaphragm continue to increase their food intake in response to fourth ventricle

injection of MK-801 [46]. This result might seem to suggest that vagal afferents are not necessary for MK-801's effect on food intake. However, it is important to remember that section of the vagus distal to the nodose ganglion does not eliminate vagal terminals from the NTS. These terminals survive (Fig. 5B), and vagal afferents actually attempt to reconnect with their peripheral targets [47]. Only removal of the nodose ganglion eliminates all vagal afferent terminals from the NTS. However, bilateral nodose extirpation is lethal. Therefore, in an attempt to assess NMDA receptor antagonist effects in a preparation lacking central vagal afferent terminals we performed unilateral nodose removals [46]. This produced rats in which vagal afferent terminals were intact on one side of the hindbrain but absent on the other (Fig. 5A). We then implanted cannulas either ipsilateral or contralateral to nodose removal and tested for increased food intake in response to injection of MK-801, comparing responses to antagonist injections via cannulas on the side with intact vagal afferent terminals to response to antagonist on the side where afferent terminals were absent. Intriguingly, injections on the side contralateral to nodose removal (vagal terminals intact) were associated with increased intake, while MK-801 infused ipsilateral to nodose removal (terminals absent) did not increase food intake (Fig. 5A'). As an additional control we cut the vagal trunk just below the nodose on one side (Fig. 5B) and compared the effects of MK-801 infused ipsilateral or contralateral to the unilateral subnodose vagotomy. In this case vagal afferent terminals persist both on the operated and unoperated side, and indeed both ipsilateral and contralateral MK-801 infusions increased food intake (Fig. 5B').

At present, the results from our experiments with unilateral nodose removal are the best evidence we have to suggest that NMDA receptors on vagal afferents themselves participate in the control of food intake. However, work in progress is yielding interesting leads that are consistent with presynaptic vagal NMDA receptor participation in vagally mediated reduction of food intake. Specifically, we have been examining the effect of NMDA receptor antagonism on signaling cascades that are activated during vagally mediated satiation. Previously, Berthoud and coworkers [48] reported that CCK injection increased phosphorylation of extracellular signal-regulated kinases 1 and 2 (pERK) in NTS neurons. Moreover, these investigators were able to attenuate CCK-induced reduction of food intake with the MEK inhibitor, UO-126. Several recent reports indicate that neuronal ERK1/2 phosphorylation in spinal primary afferents, as well as spinal dorsal horn neurons, is dependent on NMDA receptor activation [49-52]. Therefore, we have recently begun to examine the interaction of NMDA receptor activation with this signaling pathway in the NTS. We have found that CCK-induced phosphorylation of ERK1/2 in the NTS is abolished by NMDA receptor antagonist doses that prevent reduction of feeding by CCK (Campos and Ritter, unpublished). While it is clear that CCK induces pERK in intrinsic NTS neurons, our immunohistochemical results clearly indicate extensive ERK phosphorylation in the NTS neuropil. Further, dual label staining for pERK and markers of vagal afferent endings reveals that CCK induces pERK in vagal afferent fibers in the NTS, which is attenuated or prevented by NMDA receptor antagonist.

While we are continuing intensive efforts to dissect the potential roles of pre- and post-synaptic NMDA receptors in the NTS in the control of food intake by CCK and other satiation signals, it is important to realize that we would not want to rule out a contribution of peripheral NMDA receptors in control of food intake. In this regard it is especially interesting that the cellular machinery for glutamatergic neurotransmission and NMDA receptor function exists in the peripheral vagal/gastrointestinal axis. For example, several cell types in the wall of the GI tract appear to be equipped for vesicular release of glutamate. Specifically, subpopulations of intrinsic enteric neurons express immunoreactivity for vesicular glutamate transporter 2 (VGLUT2) [53]. The proximity of such intrinsic neurons and terminals to vagal afferent endings, such as IGLEs, is consistent with interaction of enterically released glutamate and vagal afferent NMDA receptors. Vesicular glutamate

transporter also has been identified in enteroendocrine-type cells, which secrete peptides that contribute to the control of food intake [54]. Therefore, endocrine mucosal cells could supply glutamate, as well as peptide, that could act on nearby vagal afferent endings. Finally, a report by Slattery et al. [31] has reported that gastric mechanoreceptive signaling, recorded from single, decentralized, mouse gastric afferent fibers in vitro, is enhanced by NMDA agonist application and attenuated by NMDA receptor antagonist application. These results suggest that release of glutamate from GI sources might modulate vagal afferent activity, at least in part, via action at peripheral vagal afferent NMDA receptors.

Two lines of preliminary investigation from our own laboratory are consistent with possible peripheral NMDA receptor contributions to control of food intake. First, we have identified a subpopulation of myenteric neurons that express VGLUT2 immunoreactivity. These VGLUT2 positive neurons express increased Fos immunoreactivity following CCK injection. Therefore, it is conceivable, though by no means proved, that they release glutamate when activated by CCK. Considering the results reported by Slattery et al. [31], it is appealing to speculate that glutamate from enteric neurons could modulate vagal afferent signaling triggered by other gastrointestinal stimuli, such as CCK. Additional support for a contribution of peripheral NMDA receptors in control of feeding comes from preliminary experiments in which we compared effects of food intake when rats were infused with MK-801 via either jugular venous catheters or aortic arterial catheters [55]. The tips of our aortic arterial catheters resided just cranial to the celiac arterial juncture with the aorta (near celiac arterial). Therefore, the arterial infusions enabled MK-801 to arrive in the gastrointestinal vascular bed at relatively higher concentrations than when the same infusion was made via a jugular catheter. With non-food restricted rats, adapted to consume a 15% sucrose meal, we found that MK-801 infused via the “near celiac” catheter increased intake at antagonist doses that were less effective when infused via a jugular catheter (Fig. 6).

In conclusion information relayed to the brain by vagal afferent neurons comprises an important contribution to the process of satiation. Glutamate is a major transmitter of vagal afferent neurons, and fast excitatory transmission from vagal afferents to second order neurons in the NTS appears to be mediated by glutamate. Antagonism of NMDA-type glutamate receptors in the NTS increases food intake, seemingly by delaying satiation and extending the duration of meals. Although, the cellular location(s) of NMDA receptors that participate in satiation is still uncertain, attenuation of satiation by NMDA receptor antagonists may be due, at least in part, to their action on primary vagal afferents themselves. While evidence is accumulating that NMDA receptors located on vagal afferent endings in the hindbrain may be involved in control of food intake, there also is preliminary evidence that peripheral glutamate also might influence food intake. Fig. 7 illustrates a hypothetical model illustrating the anatomical connections by which NMDA receptor expression on vagal afferents could provide a parsimonious mechanism by which glutamate could modulate satiation signals both at their point of origin in the GI tract and at the portal through which they are received in the central nervous system.

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Figure 1.

Left panels: Photomicrographs of 10 micron sections from rat nodose ganglia illustrating vagal afferent neurons labeled with affinity-purified polyclonal primary antisera raised in goat against the NR1 or NR2C NMDA channel subunits. Right panel: Histogram depicting the proportion of the total vagal afferent population immunopositive for various NMDA channel subunits. Data replotted from Czaja et al., 2006 (1). Note that more than 95% of vagal afferents express NR1 immunoreactivity. The NMDA channel is heteromeric containing both NR1 and NR2 subunits. However, NR1 is obligatory for all NMDA receptors. The fact that it is expressed in most, if not all, vagal afferents suggests that all vagal afferents express NMDA receptors.

Figure 2.

Sucrose intake by 16h food deprived rats offered 15% sucrose solution. The non-competitive NMDA channel blocker, MK-801 (100 micrograms/kg, IP), significantly increased sucrose intake. Redrawn from Burns and Ritter, 1997 (33).

Figure 3.

Twenty-five min intake of 15% sucrose by 16h food deprived rats. The non-competitive NMDA channel antagonist, MK-801, was injected IP after the rats were allowed to consume sucrose for 5 min (A), 10 min (B) or 15 min (C). After a delay of 15 additional min, during which time sucrose access was withdrawn, the rats were again given access to the sucrose tubes from 25 min. Note that when rats had consumed sucrose for 15 min, the length of a typical deprivation induced meal, MK-801 did not increase intake. However, if MK-801 was administered prior to (D), or soon after the start of the meal (A) intake was increased by MK-801. These results indicate that MK-801 does not trigger meal initiation. Rather it delays the process of satiation, which begins during the meal. Data is from Burns et al., 1998 (34).

Figure 4. Reduction of pelleted rodent diet intake by IP CCK-8 (2 micrograms/kg) in 16h food deprived rats. NTS injection of MK-801 (500ng) prevented CCK-8-induced reduction of intake, whereas injection of the same dose subcutaneously had no effect.

Figure 5.

A. Diagrammatic illustration of degeneration of central and peripheral vagal fibers and terminals following unilateral nodose ganglion removal. Note that central terminals degenerate ipsilateral but not contralateral to ganglion removal. **B.** Diagrammatic illustration showing that when vagus is sectioned distal to the nodose peripheral vagal fibers and terminals degenerate ipsilateral to nerve cut, while central terminals are not destroyed either ipsilateral or contralateral to vagal section. **A'.** 30-min intake of 15% sucrose following unilateral 50-nl injection of MK-801 or vehicle into the NTS either contralateral or ipsilateral to unilateral nodose ganglion removal. Seven of 10 injected contralateral to nodose removal increased their intake in response to MK-801, while 3 did not. Average %increase in intake of sham-operated rats after MK-801 injection did not differ from the average %increase of the 7 rats that did increase their intake in response to contralateral MK-801 injection. Even when intakes of all 10 rats injected contralateral to nodose removal were averaged, the MK-801-induced increase in intake did not differ from the %increase of sham-operated rats. None of the 10 rats injected ipsilateral to nodose ganglion removal increased their intake in response to MK-801. **B'.** 30-min intake of 15% sucrose following unilateral 50-nl injection of MK-801 or vehicle into the NTS either contralateral or ipsilateral to unilateral sub-nodose vagotomy. MK-801 increased food intake regardless of whether the injection was made ipsilateral or contralateral to vagal section. There was not difference in intake between intakes of sham-operated or vagotomized rats. These results indicate that increased food intake following central MK-801 administration depends on intact central but not peripheral vagal afferent terminals. Data replotted from Gillespie et al., 2005 (46).

Figure 6.

Percent increase of 15% sucrose intake following 5min infusion of MK-801 via a near celiac arterial catheter or jugular venous catheter. Note that at the 200ng MK-801 dose sucrose intake was increased by near celiac MK-801, but not by jugular MK-801 infusion.

Figure 7.

An anatomical model of modulation of vagal afferent signals by NMDA receptors located on or near both central and peripheral vagal afferent endings. NMDA receptor activation in vagal afferent endings in the brain or gut could be activated by glutamate released at axoaxonal synapses or might function as autoreceptors for glutamate released by vagal afferents themselves. In either case it is proposed that the activation of NMDA receptors would increase vagal afferent excitability and increase transmitter release from the vagal afferent terminals in the NTS.