Receptors and Transmission in the Brain-Gut Axis
II. Excitatory amino acid receptors in the brain-gut axis

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Hornby, Pamela J. Receptors and Transmission in the Brain-Gut Axis. II. Excitatory amino acid receptors in the brain-gut axis. Am J Physiol Gastrointest Liver Physiol 280: G1055–G1060, 2001.—In the last decade, there has been a dramatic increase in academic and pharmaceutical interest in central integration of vago-vagal reflexes controlling the gastrointestinal tract. Associated with this, there have been substantial efforts to determine the receptor-mediated events in the dorsal vagal complex that underlie the physiological responses to distension or variations in the composition of the gut contents. Strong evidence supports the idea that glutamate is a transmitter in afferent vagal fibers conveying information from the gut to the brain, and the implications of this are discussed in this themes article. Furthermore, both ionotropic and metabotropic glutamate receptors mediate pre- and postsynaptic control of glutamate transmission related to several reflexes, including swallowing motor pattern generation, gastric accommodation, and emesis. The emphasis of this themes article is on the potential therapeutic benefits afforded by modulation of these receptors at the site of the dorsal vagal complex.

ionotropic glutamate receptor; metabotropic glutamate receptor; feeding; emesis; central vagal control; gastric fundus; vagal motor nucleus

ACADEMIC AND PHARMACEUTICAL interest in the brain-gut axis has increased dramatically in the last 10 years. Information gained from studies on hindbrain neurocircuitry of sensory/motor pathways to the gut has provided insight in many areas of clinical relevance, including gastroesophageal reflux disease, dysphagia, emesis, functional dyspepsia, food intake, stress, and irritable bowel syndrome. Central vagal processing governs many normal upper gastrointestinal (GI) reflexes, such as swallowing, and those in response to distension, acidity, fat, and nutrient composition in the gut. The purpose of this article, which is part of a series on the potential for novel therapies in the brain-gut axis, is to focus on excitatory amino acid (EAA) transmission in the center of integration of the brain-gut axis, the dorsal vagal complex. Excitatory and inhibitory pathways are often complementary; therefore, some aspects of GABA neurotransmission are included, although this topic is covered in detail elsewhere in this series.

GLUTAMATE IN THE BRAIN-GUT AXIS

Numerous lines of evidence (in vitro dialysis, electrophysiological slice, functional in vivo and immunocytochemical data) all support the notion that glutamate is a primary afferent neurotransmitter of the vagus nerve, although a few studies in the 1980s countered this notion. This neurotransmitter is integral to central processing of GI reflex neurocircuitry in the dorsal vagal complex. For example, high levels of glutamate are present in labeled vagal afferent terminals, compared with the surrounding neuropil (26) of the nucleus tractus solitarii (NTS), and glutamate immunoreactivity is present in ~60% of nodose ganglion cells (23). In addition, vagal (solitary tract) stimulation in a slice preparation results in excitatory postsynaptic potentials in all neurons sampled by whole cell patch clamp, indicating that the afferent input is exclusively excitatory (25). Finally, gastric distension increases the firing of NTS neurons recorded extracellularly, and this response is inhibited by local microinjection of a broad-spectrum glutamate receptor antagonist, kynurenic acid (16).

In vagal afferent-vagal efferent (vago-vagal) GI reflexes, it is thought that the glutamate-containing afferents synapse with inhibitory GABAergic second-order neurons in the NTS (Fig. 1). These second-order neurons tonically inhibit the firing of cholinergic excitatory preganglionic motoneurons in the dorsal motor nucleus of the vagus (DMN). Thus L-glutamate stimulation of the NTS inhibits gastric motor function in vivo (20), probably because of GABA_A receptor-evoked inhibitory postsynaptic potentials in the DMN (27). Preganglionic neurons in the DMN innervate the smooth muscle of the lower esophageal sphincter (LES), stomach, small intestine, and cecum/proximal...
colon, and L-glutamate microinjection into the DMN generally excites upper GI functions.

However, not all neurons in the DMN simply drive cholinergic excitatory input to the GI smooth muscle. There is also a separate population of cholinergic preganglionic neurons that input onto nonadrenergic, noncholinergic (NANC) inhibitory motoneurons in the upper GI tract. L-Glutamate stimulation of these neurons in the DMN caudal to the obex (a surface landmark that marks where the central canal opens into the fourth ventricle) decreases upper GI motor activity (22). This emerging concept has required a rethinking of the vago-vagal reflex involving a simple chain of excitatory (glutamate)-inhibitory (GABA)-excitatory (cholinergic-cholinergic) neurons because there must be a balance of tonic inhibition of both vagal excitatory and vagal NANC neurons (Fig. 1). Thus pharmacological modulation of glutamate release at the primary afferents will ultimately alter the activity of both vagal excitatory and vagal NANC pathways.

There are several potential therapeutic applications of modulating vago-vagal reflexes involving the stomach. For example, one approach is to pharmacologically reduce inappropriate LES relaxation in response to gastric distension, which is thought to underlie the development of gastroesophageal reflux disease. Another example is to improve gastric accommodation in response to a meal in patients with functional dyspepsia, who exhibit early satiety, nausea, and vomiting. Conversely, slowing down gastric emptying and decreasing gastric accommodation may help to reduce meal size in obese patients. Finally, some of the newer antiemetics are thought to be effective because they reduce vagal afferent input into the NTS. The remainder of this article reviews the basic studies on EAA receptor modulation in the dorsal vagal complex that could potentially be exploited therapeutically. However, to interpret some of the studies, it is first necessary to review briefly the organization of the dorsal vagal complex.

There is a viscerotopic organization of both the DMN and NTS, although this viscerotopy is more pronounced for the NTS (Fig. 2). Afferent gastric fibers terminate primarily in the subnucleus gelatinosus and also in the medial and commissural subnuclei of the NTS (21). Cardiovascular afferents terminate predominantly within the medial NTS. In contrast, esophageal afferents terminate within the subnucleus centralis, and buccopharyngeal afferents terminate in the interstitial and intermediate subnuclei. Information from premotor neurons in the centralis, interstitialis, and intermediate subnuclei of the NTS is relayed to various regions of the nucleus ambiguous, where motoneurons innervate the esophagus, pharynx/cricothyroid, and intrinsic laryngeal muscles. The concentration of premotor neurons in the subnucleus centralis is such that focal stimulation in this region evokes swallowing (11). However, even though there are separate targets for esophageal and buccopharyngeal afferents in the NTS, there is the opportunity for extensive synaptic contact between the premotor (second order) neurons receiving this input. Thus the NTS is critical for the coordination of swallowing, and disruption of these connections may result in dysphagia. Within the DMN there is a looser viscerotopic organization. Neurons innervating the cecum/proximal colon are concentrated in the lateral tip, and there is a medial-lateral gradient reflecting the regions of the stomach. However, this is certainly not a rigid organization, and there may be a substantial coinervation by DMN neurons of GI regions, especially those regions that perform similar functions.

Overall, the viscerotopic organization of the dorsal vagal complex can be helpful in interpreting the potential function of receptors that are differentially distributed within it, and care can be taken in experiments to manipulate a subnucleus of relevance to a functional...
response. However, one must always use a degree of caution to prevent overinterpreting these “maps” of the gut in the brain.

**IONOTROPIC GLUTAMATE RECEPTORS AND THE BRAIN-GUT AXIS**

Ionotropic glutamate (iGlu) receptors are ligand-gated ion channels comprising of three subtypes, N-methyl-D-aspartate (NMDA), amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA), and kainate (KA) receptors.

NMDA receptors are heteromeric pentamers with subunits that are the products of two gene families, an NR1 gene and four NR2 genes. Although NR1 alone can provide a functional receptor, it is thought that NR2 increases the activity of the channel. Ultrastructural analysis of NR1 immunoreactivity in vagal afferents in the NTS demonstrates that over one-third of identified vagal afferent axons and terminals contain NR1 labeling and almost one-half of the dendrites are contacted by vagal afferent terminals containing NR1 (1). Thus NR1 modulates afferent release and is also expressed by second-order neurons in the NTS.

A specific example of NMDA receptor-mediated fast transmission relates to premotor control of swallowing. In situ hybridization for NR1 mRNA reveals a high level in the subnucleus centralis of the NTS (2), and NMDA microinjection into the NTS induces swallowing, which is blocked by the selective NMDA antagonist DL-2-amino-5-phosphonovalerate (11). Thus the release of glutamate by afferents arising from the esophagus is rapidly transferred by NMDA receptor activation to initiate the central program generator for swallowing.

The idea that these NMDA receptors in the NTS may also play a role in control of food intake has been tested in several ways. Blockade of NMDA receptors with systemic or intra-NTS microinjection of dizocilpine, a noncompetitive NMDA antagonist, delays satiation and increases meal size (3, 28). To study the neurocircuitry underlying this phenomenon, Zheng and colleagues (30) studied the effect of dizocilpine infusion into the fourth ventricle on the activation of c-fos by physiological gastric distension. NMDA blockade significantly reduced c-fos expression evoked by gastric distension in some NTS subnuclei, such as the commissural, gelatinous, and dorsomedial subnuclei. However, dizocilpine did not significantly prevent gastric distention-induced c-fos throughout the NTS overall. Because the investigators were careful to provide a low-threshold physiological stimulus, which is unlikely to be nociceptive, these results do not lend support to the idea that NMDA receptor plays a major role in mechanoreceptor signaling in the NTS (30). Indeed, a later study showed that NMDA blockade accelerated gastric emptying, which itself could indirectly alter gastric mechanoreceptive afferent signaling and would result in larger meal size (5).

In addition to the role of NMDA receptors in modulation of vago-vagal reflexes in the dorsal vagal complex, there is also evidence for participation of non-NMDA receptors in this region. For example, by recording neuronal vagal efferent discharge and providing different forms of vagal afferent stimulation, it was reported that a non-NMDA receptor antagonist reduced efferent vagal responses by 65% (18). The results indicated that central non-NMDA receptors are involved in mediating a wide range of upper GI mechanoo- and chemosensitive afferent inputs onto vagal efferents. It is not possible to determine the precise site of action of the non-NMDA antagonists in these experiments; however, whole cell patch-clamp recordings in brain slice preparations showed that NTS excitatory postsynaptic potentials, which were evoked by...
solitary tract stimulation, were blocked by non-NMDA antagonists (25). In addition, NTS stimulation activated non-NMDA (and NMDA) postsynaptic receptors in DMN neurons (29). Thus non-NMDA receptor-mediated events in response to afferent glutamatergic signaling occur at several levels, including the level of second-order NTS neurons and at the level of DMN vagal motor output.

Unfortunately, pharmacological characterization cannot distinguish between AMPA and KA receptors. For example, non-NMDA antagonists such as 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(f)quinoxaline (NBQX) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) are not selective for AMPA receptors. However, the cloning of the AMPA receptor has provided probes for the detection of the subunits in the dorsal vagal complex. The AMPA receptor is made up of various combinations of four subunits termed GluR1–4. GluR2 immunostaining is moderately dense in the medial NTS and interstitial subnucleus, whereas GluR2/3 immunolabeling is present throughout the entire dorsal vagal complex (10). Consistent with the expression of these AMPA subunits, CNQX inhibited swallowing that was evoked by L-glutamate microinjection in the NTS (11).

In a different study, extracellular firing in the subnucleus centralis in response to esophageal distension was significantly reduced by CNQX (15). In brain stem slice preparations, electrical stimulation of the solitary tract resulted in excitatory postsynaptic potentials that were blocked by the same antagonists (15). Thus these data, together with the discussion of NMDA receptors in the swallowing circuits above, lead to the conclusion that both NMDA and non-NMDA receptors are activated by stimulation of primary afferents to the subnucleus centralis and other regions of the NTS. Interestingly, the excitatory input is also balanced by a tonic inhibitory GABAergic drive onto premotor neurons, and it is thought that release from this inhibition unleashes an excitatory swallowing drive.

Several studies have assessed the potential therapeutic applications of blockade of iGlu receptors in the prevention of emesis. Systemic blockade of non-NMDA receptors effectively inhibited cisplatin-induced emesis in ferrets (6), and NBQX applied directly into the fourth ventricle abolished the increase in salivary secretion and fictive retching (7). In contrast, NMDA blockade with dizocilpine was ineffective (7). Another study reports that NMDA receptor antagonists may provide a modest inhibition of cisplatin-induced emesis, but this occurs at doses that cause other behavioral effects such as sedation (13). Hence it appears that non-NMDA receptor antagonists are more critical than NMDA receptor antagonists for a potential antiemetic effect.

Finally, with regard to control of vagal preganglionic motor outflow, non-NMDA receptors such as AMPA GluR2 are highly expressed in neurons of the DMN (12). Activation of both non-NMDA and NMDA receptors in the DMN in vivo increases gastric contractility, and this effect was blocked by the appropriate antagonists (24). These neurons are normally under tonic GABAergic inhibition because microinjection of bicuculline, a GABA A receptor antagonist, unveils a strong excitatory drive to DMN motoneurons, which can be blocked by a broad-spectrum iGlu antagonist, kynurenic acid. Modulation of the level of tonic activation of vagal excitatory and/or inhibitory NANC preganglionic neuron output can contribute to therapeutic strategies for GI disorders. For example, systemic administration of baclofen, a GABA B receptor agonist, reduces the incidence of transient LES relaxations (which are associated with reflux events) in response to gastric insufflation. This effect is probably caused by both postsynaptic GABA B receptor inhibition at the level of the DMN and presynaptic GABA B receptor inhibition of the peripheral vagal efferent terminals (17). Therefore, blockade of iGlu receptors expressed by vagal preganglionic neurons could be regarded as a means to alter vagal efferent function. However, to my knowledge, this has not been assessed with regard to potential therapeutic applications.

In conclusion, iGlu receptors in the dorsal vagal complex have been implicated in several aspects of the brain-gut axis. These receptors are integral to synaptic transfer of information from primary afferents. The strongest evidence for their role in the brain-gut axis lies in the fast transmission of the swallowing pattern generator at the level of the subnucleus centralis. However, in areas of therapeutic interest, such as control of food intake and emesis, modulation of iGlu receptors in the NTS has not led to spectacular results. In light of the cancellation of several pharmaceutical programs that were developing iGlu receptor antagonists to prevent neuronal excitotoxicity, the side effects of iGlu antagonists make it seem unlikely that these would be clinically beneficial in the brain-gut axis.

**METABOTROPIC GLUTAMATE RECEPTORS AND THE BRAIN-GUT AXIS**

Although it was initially thought that glutamate acted only on iGlu receptors, it was later discovered that this ubiquitous transmitter also acts on metabotropic (mGlu) receptors. The cloning of mGlu receptors revealed that they have sequence homology with GABA B receptors in G protein-coupled receptor family 3. There are three groups of mGlu receptors based on their sequence similarity. Group I (mGlu1 and mGlu5) receptors activate phospholipase C, whereas group II (mGlu2 and mGlu3) and group III (mGlu4, mGlu6, mGlu7, and mGlu8) receptors inhibit adenyl cyclase activity. Group I receptors generally increase cell excitability by inhibiting K+ channels and are mostly postsynaptic, although presynaptic effects have also been reported. Group II and III receptors are mostly on glutamatergic terminals and inhibit neurotransmitter release. Although progress has been made in localizing these receptors in the brain, a problem associated with studying their physiological function is the lack of selective agonists and antagonists, which is reviewed elsewhere (19).
In light of the putative autoreceptor function of group II and group III mGlu receptors, one would predict that these would modulate function on glutamatergic vagal afferent input to the NTS. A series of studies have looked at this by using different frequencies of electrical stimulation of the vagus, and the results support the idea of a mGlu autoreceptor at the level of the vagal afferents in the NTS. Following high-frequency electrical stimulation of vagal fibers, action potentials that were recorded extracellularly in the NTS were augmented by exposure to α-methyl-(4-phosphonophenyl)glycine (MPPG; group II and III antagonists). However, increasing afferent input to the NTS (by high-frequency stimulation) is also associated with a reduced postsynaptic response, which could be caused by desensitization of postsynaptic iGlu receptors rather than presynaptic modulation of glutamate release. This possibility was rejected because cyclothiazide, an agent that inhibits AMPA receptor desensitization, did not affect the synaptic depression observed at higher stimulation frequencies (4). In addition, Glaum and Miller (8), recording from neurons in an NTS slice preparation, found that (1S,3R)-1 aminocyclopentane-1,3-dicarboxylate (ACPD; an agonist with affinity for all 3 groups of mGlu receptor) reduced the amplitude of neuronal responses evoked by solitary tract stimulation. Therefore, all of these studies concur that group II/III mGlu receptors can modulate afferent vagal release. In support of these functional data, group III mGlu7 receptor immunoreactivity is detected within almost all neuronal cell bodies in the nodose ganglion (14). Overall, the data indicate that mGlu autoreceptors can exert quite powerful effects on presynaptic glutamate release from primary vagal afferents. However, to my knowledge, there are no reports on the functional effects of these agents in the NTS to modulate GI vago-vagal reflexes.

On the basis of their immunocytochemical distribution, there is a strong indication that mGlu receptors are involved in the swallowing program generator modulation. For example, mGlu1a and mGlu2/3 receptors immunostain fibers and terminals within the intermediate and interstitial subnuclei (9). Another group I receptor, mGlu5, is noted in fibers/terminals in the intermediate subnucleus. These data suggest that group I and II mGlu receptors are associated with premotor neuron control in regions in which laryngeal and pharyngeal afferents terminate. Another intriguing finding is that the group III mGlu7 receptor is highly expressed in the subnucleus gelatinosus. This a region in which gastric vagal afferents directly contact with vagal efferents (21), and the fact that these are probably autoreceptors could allow for modulation of this monosynaptic reflex. However, this interesting possibility remains to be tested in functional studies. Finally, in terms of vagal motor output, mGluR1a receptor immunoreactivity is prevalent throughout the compact formation of the nucleus ambiguous and within the DMN. This suggests that postsynaptic mGlu receptors modulate motoneuron output to GI striated and smooth muscle.

In conclusion, pharmacological manipulation of mGlu receptors in the NTS demonstrates that these receptors are functional in the neurocircuitry of this region. Therefore, there is every reason to expect that these receptors can also modulate swallowing motor pattern generation and smooth muscle GI reflexes in this region, especially because mGlu receptors are expressed in the appropriate regions of the NTS and DMN. Because the group II/III mGlu receptors are autoreceptors, pharmacological modulation of these receptors could alter vagal afferent signaling and provide potential therapeutic avenues related to nausea and emesis or meal size. However, at the present time there is insufficient published information on the role of mGlu receptors and vago-vagal reflexes in the dorsal vagal complex to make accurate predictions about their potential role.

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REFERENCES


