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Astrocytes in the hindbrain detect glucoprivation and regulate gastric moti

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ABSTRACT

Glucoprivation is a strong signal for the initiation of gastrointestinal contractions. While this relationship between utilizable nutrient levels and gastric motility has been recognized for more than 100 years, the explanation of this phenomenon has remained incomplete. Using widely differing approaches, recent work has suggested that the hindbrain is responsible for this chemoreflex effect. Surprisingly, astrocytes may be the main glucodetector elements under hypoglycemic conditions. Our own work using in vitro live cell calcium imaging shows that astrocytes in the NST increase cytoplasmic calcium in a concentration dependent manner in reaction to reductions in glucose. This effect is reversed on restoration of normal glucose concentrations. In vivo single unit neurophysiological recordings show that brainstem neurons driving gastric motility are activated by glucoprivic stimuli. Studies in intact animals verify that both dorsal medullary and systemic glucoprivation significantly increases gastric motility. Astrocyte inactivation with fluorocitrate blocks the pro-motility effects of glucoprivation. Thus, it appears that intact astrocyte signaling may be essential to glucoregulatory control over gastric motility.

1. Introduction

Nutrient levels, feeding behavior, and gastric motility have been linked in a correlational sense for more than 100 years. The relationship was heralded by William Beaumont's direct observations of the digestive processes of Alexis St. Martin (Beaumont, 1833). Pavlov next observed that increases in gastric motility and secretion were related to periods of food deprivation and the anticipation of eating. This led to Pavlov's proposal of a "cephalic phase" of digestion whereby stimuli, both internal [e.g., glucoprivation] and external [e.g., cues related to the expectation of feeding], were integrated by the brain to augment digestion in advance of feeding (Pavlov, 1910). In 1912, Cannon and Washburn connected food deprivation and the sensation of gastric contractions with the urge to eat (Cannon and Washburn, 1912). Classic studies from the 1920s through the 1990s (Bulatao and Carlson, 1924; Richter, 1941; Novin et al., 1973; Cato et al., 1990) have gone on to establish that glucoprivation is a robust signal for the initiation of feeding and perhaps the strongest known stimulant of gastric motility.

Given the crucial importance to life of glucose availability, it is not surprising that there is duplication of glucodetectors in both the periphery as well as within the central nervous system. The loci of glucodetection important to homeostasis are still a matter of controversy, though there is ample evidence for hepatic vagal, solitary nucleus, ventral medullary and hypothalamic sensors (Novin et al., 1973; Borg et al., 1999; Ritter et al., 2000). This distributed sensor/regulator arrangement is observed for other critical physiological parameters such as fluid volume and osmolality (Antunes-Rodrigues et al., 2004; Simard and Nedergaard, 2004) and blood gases (Bianchi et al., 1995; Gourine and Kasparov, 2011).

The nucleus of the solitary tract [NST] is the most likely site for the convergence of glucodetection and the regulation of gastric motility. Several reports provide evidence for the NST as a gluco-sensory structure (Ritter et al., 2000; Marty et al., 2005) that maintains efferent connections necessary for regulating nutrient homeostasis and digestion (Mizuno and Oomura, 1984; Adachi et al., 1995; Yettefti et al., 1995; Dallaporta et al., 1999, 2000; Rogers and Hermann, 2012). Additionally, the NST is the recipient of vagal afferent projections from the gut. The NST integrates this vagal input and controls gastric motility and secretion via short axon projections to the immediately subjacent dorsal motor nucleus of the vagus [DMN]. Most of these gastric "vago-vagal" motility control reflexes are inhibitory [for example, see Fig. 1]; the result of the withdrawal of tonic excitatory vagal efferent inputs to the stomach. Additionally, vagal afferents from the gut can activate a parallel "non-adrenergic, non-cholinergic" [NANC] efferent path that causes the active inhibition of gastric smooth muscle. This NANC path is probably composed of vagal afferents that activate noradrenergic neurons in the NST that, in turn, activate the NANC path neurons in the DMN. These NANC-DMN neurons project to the gastric enteric plexus where they activate nitregic or adrenergic neurons to cause the relaxation of the stomach (Abrahamsson, 1986; Barbier and Lefebvre, 1993; Takahashi and Owyang, 1997; Rogers et al., 1999; Rogers and Hermann, 2012).

Until recently, there would have been unanimous agreement that the cells responsible for sensing, signaling, and transmitting data concerning glucose availability are neurons. However, a highly controversial and

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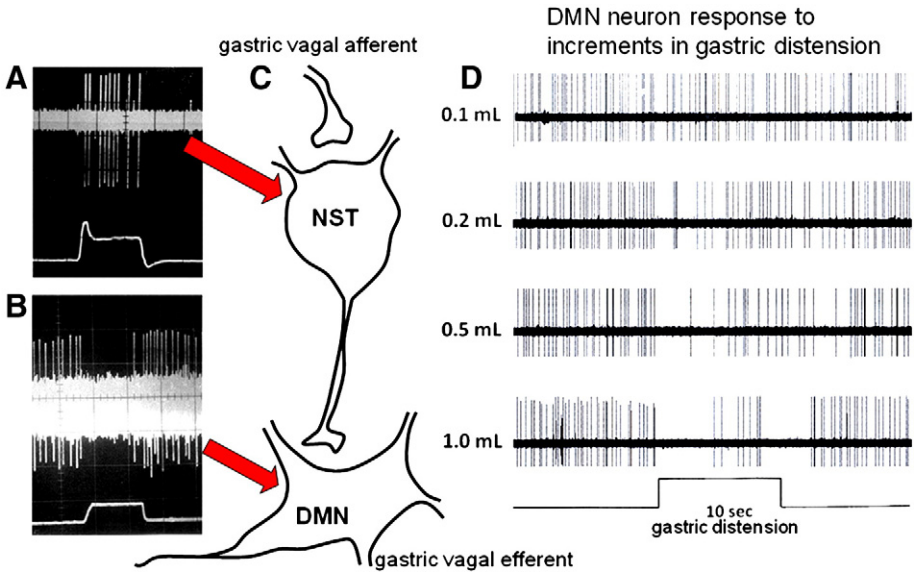

Fig. 1. In vivo electrophysiological recordings identifying gastric-related second order visceral sensory neurons in the nucleus of the solitary tract (NST) [A] and parasympathetic efferent neurons in the dorsal motor nucleus (DMN) [B] responding to mild distension (0.5 mL) of a balloon in the gastric antrum (from McCann and Rogers, 1992). Note the characteristic, spontaneous, pacemaker activity of the DMN neuron that is interrupted by distension of the stomach (lower trace). [C] Schematic representation of the vago-vagal reflex connection responsible for the gastric accommodation reflex whereby antral distension produces relaxation of the corpus through removal of tonic vagal efferent cholinergic excitation. DMN neuronal responses to incremental changes in gastric inflation [D] show that the reflex is quite sensitive; the threshold for initiation of the reflex in the rat is approximately 0.1–0.2 mL distension.

provocative paper by Nell Marty et al. (Marty et al., 2005) has thrown the primacy of neuronal glucodetection into question in favor of the astrocyte.

1.1. Astrocytes and integrated brain function

The classic neurophysiological role for the astrocyte in the control of CNS function has been seen as passive and supportive. In this view, the astrocyte controls the extracellular nutrient, metabolite, and ionic environment for the neuron, while also working to dispose of and recycle released neurotransmitters and their break-down products (Volterra and Meldolesi, 2005). While astrocytes certainly perform these important functions, recent neurophysiological studies in mixed neural–glial culture suggest that astrocytes are critical regulators of neuronal excitability and synaptic efficiency (Haydon and Carmignoto, 2006). Astrocytes possess a broad array of receptors for neurotransmitters, hormones, cytokines, and other agonist peptides. Agonist inputs to these astrocytic receptors can cause dramatic increases in cytoplasmic calcium (Haydon and Carmignoto, 2006), which, in turn, are coupled to astrocytic release of a variety of neuroactive substances, especially glutamate and ATP.

Agonist-induced calcium signals in astrocytes can cause the delayed activation [or inhibition] of adjacent neurons (Parpura and Haydon, 2000). Neurophysiological studies in neuronal–glial co-cultures suggest, for example, that agonists acting primarily on astrocytes can generate a delayed activation signal in neurons as a result of a calcium-induced exocytosis of glutamate from the glia (Parpura and Haydon, 2000). Depending on the circuitry under study, astrocytic glutamate release can affect neuronal excitability through action on metabotropic, NMDA, and/or AMPA receptors (Haydon and Carmignoto, 2006). Again, depending on the specifics of the circuitry, these astrocyte-glutamate mediated effects can increase or decrease the post-synaptic sensitivity of neurons to presynaptic input (Fellin et al., 2006). While there is strong evidence for astrocytic glutamate release to exert significant effects on neuronal function, recent studies show that similar effects can also be produced by astrocytic release of other agonists including serine, ATP, or nitric oxide (Haydon and Carmignoto, 2006).

Astrocytes are also implicated in the presynaptic regulation of afferent neuronal signal traffic. Glial release of ATP could act at a variety of pre-synaptic purinergic receptors to produce a range of circuit-specific effects (Jin et al., 2004; Burnstock et al., 2011). In particular, ATP can act at P2X3 receptors on vagal afferents within the NST to provoke significant presynaptic glutamate release [(Jin et al., 2004; Shigetomi and Kato, 2004; Rogers et al., 2006a, 2006b); see also "Youtube" movie, search: "K5RCR", see movie of ATP effects on vagal afferents]. In an apparent contradiction, glial release of ATP can also produce both pre- and post-synaptic inhibition following the ectoenzyme conversion of ATP to adenosine, an A1 receptor agonist (Newman, 2003). It is also possible that astrocytic release of glutamate may have significant effects at presynaptic terminals via activation of NMDA and metabotropic receptors (Page et al., 2005; Czaja et al., 2006).

Just as astrocytes can release gliotransmitters that alter neural circuit function, a plethora of neuronal afferent transmitters including norepinephrine, glutamate, GABA, acetylcholine, histamine, adenosine and ATP (Haydon and Carmignoto, 2006) can regulate calcium signaling in astrocytes. It is interesting to consider the possibility that visceral afferent inputs could influence the chemosensitivity of NST astrocytes. For example, if glia serve as important chemosensory or sentinel elements for cytokines, chemokines, hormones, nutrients, etc, then neural input to NST glial cells (McDougal et al., 2011) should certainly affect their sensitivity to these physiological parameters.

It seems likely that astrocytes, serving as local chemodetectors, can affect autonomic control through gliotransmitter release onto neuronal autonomic control circuitry (Hermann et al., 2009; Funk, 2010; Gourine and Kasparov, 2011; McDougal et al., 2011). It is also the case, however, that NST astrocytes receive vagal afferent inputs through AMPA receptors (McDougal et al., 2011). This mutual relationship between astrocyte and neuron opens the possibility that astrocyte chemosensitivity can regulate autonomic reflexes and can, itself, be regulated by visceral afferent inputs to the NST tracking autonomic function [Fig. 2].

1.2. Astrocyte interactions with gastric motility-regulating neurons in the brainstem

Our laboratory is interested in the relationship between disease and failure of autonomic control of digestion. Specifically, we have studied the connection between the activated immune system (as occurs in numerous disease processes) and digestive system failure as characterized by nearly absent gastric motility, nausea and vomiting (Hermann