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Highlights in basic autonomic neurosciences: Diet-induced enteric, vagal and brainstem dysfunction

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Introduction

Vagally-dependent reflexes are critical to the control and regulation of appropriate gastrointestinal (GI) functions, including early satiety and the regulation of food intake. Visceral sensory information is relayed to the central nervous system via the afferent vagus nerve, the central terminals of which enter the brainstem via the tractus solitarius and terminate on neurons of the nucleus tractus solitarius (NTS). These second-order NTS neurons assimilate and integrate this vast volume of sensory information with inputs received from other brainstem and higher CNS centres involved in the regulation of autonomic homeostatic functions. The integrated signal is then relayed to the adjacent dorsal motor nucleus of the vagus (DMV) which contains the preganglionic parasympathetic motoneurons that transfer the appropriate output response back to the upper GI tract via the efferent vagus nerve. While the enteric nervous system (ENS) is capable of a significant degree of autonomy of GI functions, the stomach and upper GI tract in particular, are more dependent upon extrinsic neural inputs, particularly through the vagus nerve.

Obesity is known to adversely impact vago-vagal reflex control of GI functions, resulting in altered gastric emptying, dysmotility, dyspepsia, discomfort and nausea as well as abnormal satiation. While this has been the topic of investigation by many groups for several years, more recently it has become apparent that diet and obesity affect all aspects of vago-vagal reflex control of GI functions and, furthermore, diet and obesity may exert distinctly different effects upon these functions.

High-fat diet and body weight have different effects on cannabinoid CB₁ receptor expression in rat nodose ganglia : N.L. Cluny, E.D. Barboir, K. Mackie, G. Burdgya, G.J. Dockray, K.A. Sharkey *Autonomic Neuroscience* (2013) 179 (X): 122-130

Article Summary

Sensory information from the gastrointestinal (GI) tract is relayed to the brainstem via vagal afferents, whose cell bodies lie in the nodose ganglia. Feeding status is well recognized to induce plasticity in the neurochemical phenotype of vagal afferent neurons, but there is still confusion and controversy regarding the distinct contributions of feeding status, and obesity. In the present study, the authors used a variety of techniques to investigate alterations in

cannabinoid receptor-1 (CB₁) expression in the nodose ganglion of rats fed *ad libitum* or food-deprived while maintained on either control diet (12% kcal from fat) or on a high fat diet (HFD; 45% kcal from fat). In control rats, CB₁ receptor mRNA levels were not affected by 24hr fasting but the profile of CB₁ immunoreactive neurons increased (when measured with an antibody directed against the N-terminal, but not the C-terminal, of the CB₁ receptor). In rats fed a HFD, regardless of weight, the number of CB₁ immunoreactive neurons was higher than in control rats but, in contrast, these levels were not increased by fasting. In rats that were fed the control diet but of similar weight to the HFD-fed rats that were resistant to weight gain, the proportion of CB₁ immunoreactive neurons was increased by fasting. These results suggest that (1) the proportion of CB₁ receptors immunoreactive to an antibody raised against the N-terminal of the receptor is depending on the feeding status, (2) differences in immunoreactivity to antibodies directed against the CB₁ N-terminal vs C-terminal reflect alterations in receptor activity rather than receptor expression, (3) CB₁ receptor immunoreactivity is enhanced by an increase in body weight and (4) HFD may attenuate the fasting induced increase in receptor expression.

Article Commentary

Vagal afferent fibers and neurons play an important role in relaying sensory information from the GI tract to the brainstem and in regulating, modulating and modifying vagally-dependent visceral functions. While it is well known that the neurochemical phenotype of vagal afferent neurons is modulated by diet and feeding status, the particular roles that diet *vs* obesity *vs* feeding status remains to be clarified. The results from this study raised several important points, not the least of which is the potential for dramatically different results depending on antibody selectivity. The authors suggest that antibodies directed against the N-terminal, rather than the C-terminal, end of the CB₁ receptor reflect receptor activity, rather than protein expression, which may explain some of the conflicting results reported to date. Immunoreactivity for CB₁ receptor was increased in rats fed a HFD, regardless of their weight. Furthermore, rats fed a control diet but weight matched to HFD-fed rats had higher expression of CB₁ receptor than their leaner counterparts suggesting that weight, rather than diet, may be responsible for the regulation of CB₁ receptor levels. This finding highlights an important potential confounder to many feeding-related studies, namely diet and obesity may exert effects through different mechanisms and the effects of one do not always correlate with the other.

Diet-dependent modulation of gastro-oesophageal vagal afferent mechanosensitivity by endogenous nitric oxide : Kentish S.J., O'Donnell T.A., Wittert G.A., Page A.J. : Journal of Physiology (2014): 3287-3310

Article Summary

Within the gastrointestinal (GI) tract, nitric oxide (NO) modulates motility, mucosal function, inflammation and blood flow in addition to modulating vagal afferent neuron excitability and responsiveness. Previous studies have suggested that the effects of NO to modulate food intake depend upon feeding status; NOS knockout mice have normal daily food intake behavior, but fail to modulate feeding in response to orexigenic or anorexigenic

neuropeptides and fail to modulate food intake in response to deprivation. In the present study, the authors used fiber recording techniques to investigate the ability of NO to modulate the mechanosensitivity of gastroesophageal vagal afferent fibers and demonstrated that, in the fed state, NO inhibits mucosal afferents via actions involving a guanylate cyclase-cGMP dependent pathway that results in reactive oxygen species (ROS) production. In fasted mice, however, NO *potentiated* the response of mucosal and mechanosensitive vagal afferents via actions involving activation of a hyperpolarization-activated cyclic nucleotide gated (HCN) channel.

Article Commentary

A significant body of work has demonstrated that vagal afferent neurons alter their neurochemical phenotype in response to feeding or fasting hence alter their responsiveness to a variety of GI neurotransmitters and neuromodulators. The present study provides additional support for the concept that GI vagal afferent fibers are subject to a significant degree of plasticity, even under physiological conditions, by demonstrating that the same neurotransmitter, NO, can have opposing effects on vagal afferents due to the recruitment of different downstream signaling pathways which, in turn, is determined by feeding status. Upon further consideration, however, it also implies that experimental methodology is of critical importance when studying vagovagal reflexes, since completely different conclusions may be drawn depending upon the time of day or feeding status of the animal at the time of experimentation and suggests that this research field would benefit from standardization of methodology which may allow more easy comparison of results across laboratory groups.

Ability of GLP-1 to decrease food intake is dependent on nutritional status : Ronveaux C.C., de Lartigue G., Raybould H.E. : Physiology and Behavior (2014) 135: 222-229

Article Summary

The GI neurohormones, glucagon-like peptide 1 (GLP-1), is released from intestinal L-cells in response to intraluminal fats and carbohydrates and activates vagal afferent fibers to induce satiation and decrease food intake. The present study used a combination of approaches to investigate the hypothesis that the expression of GLP-1 receptors (GLP-1R) on vagal afferent neurons alters in response to feeding status which, in turn, modulates the ability of GLP-1 to inhibit food intake. The authors demonstrate (1) that exogenous administration of GLP-1 decreases food intake in fed, but not fasted, rats and (2) while neither the proportion of vagal afferent neurons expressing GLP-1R, the amount of protein or mRNA for GLP-1R is altered by feeding status, the proportion of GLP-1R associated with the neuronal membrane of vagal afferent neurons is increased in fed as compared to fasted rats.

Article Commentary

Several studies from different laboratories have demonstrated that the proportion of receptors for a variety of GI neuropeptides and neurotransmitters that are present on the cell

membrane of vagal afferent neurons is open to modulation by feeding or fasting. As such, this confers an 'anorexigenic' or 'orexigenic' phenotype on vagal afferent neurons. Many of these studies demonstrated alterations in receptor localization only after relatively prolonged periods of fasting. In the present study, however, the authors demonstrate that, while feeding or fasting does not alter total GLP-1R mRNA or protein expression, it does alter the proportion of the total GLP-1R present on the neuronal membrane. In fact, co-localization of GLP-1R with the neuronal membrane increased in response to a short period of re-feeding suggesting (a) trafficking of receptors to the neuronal membrane may occur in the immediate post-prandial period and (b) such trafficking may play a physiological role in the regulation of food intake by GLP-1 and may provide some explanation as to why exogenous GLP-1 inhibits food intake only after feeding. Such findings again confirm the inherent, and physiological, plasticity within vagal afferent neuronal signaling and continue to emphasize the need for rigorous scientific control over experiments within this field of research.

Roux-en-Y gastric bypass reverses the effects of diet-induced obesity to inhibit the responsiveness of central vagal motoneurons : Browning K.N., Fortna S.R. & Hajnal A. : Journal of Physiology (2013) 591(1): 2357-2372

Article Summary

In the present study, the authors made electrophysiological recordings from vagal motoneurons to investigate whether diet-induced obesity (DIO) altered the excitability and responsiveness of vagal efferent neurons and whether these effects were reversed following Roux-en-Y gastric bypass (RYGB). Vagal motoneurons from rats fed a high fat diet for 12-14 weeks were less excitable, fired fewer action potentials and were less responsive to GI neuropeptide neurotransmitters such as GLP-1 and cholecystokinin (CCK) than age-matched control rats. All of these DIO-induced effects were reversed following weight-loss in response to RYGB, suggesting they may be due to obesity, rather than diet. DIO also affected the morphological properties of vagal motoneurons, increasing their size and dendritic arborization; RYGB did not reverse these alterations suggesting that these effects may be due to diet, rather than obesity.

Article Commentary

DIO is known to alter the properties and responsiveness of vagal afferent neurons and fibers as well as enteric neurons but few studies have investigated the effects of DIO on central vagal neuronal functions. In this study, one of the few to have investigated the effects of DIO on the properties of central neurons, the authors demonstrate that, as observed with peripheral vagal afferent neurons, DIO decreased the excitability and responsiveness of efferent vagal motoneurons. This decreased excitability appeared to be multifactorial, affecting several ionic conductances within vagal efferent neurons rendering them less excitable, as well as decreasing their ability to respond to GI neurohormones such as CCK and GLP-1. Remarkably, despite being maintained on the same diet post-surgery, the majority of DIO-induced alterations in vagal efferent neuronal function reversed following RYGB-induced weight loss. This would suggest that vago-vagal neurocircuits are able to be modulated and adapt throughout life and that long-term conditions such as obesity may not

induce permanent damage or neurocircuit rewiring. Given this apparent reversibility, vagal neurocircuits may present attractive targets for obesity research.

High-fat Ingestion correlates with Neuropathy in the Duodenum Myenteric Plexus of Obese Mice with Symptoms of Type 2 Diabetes : Chloe M. Stenkamp-Shrahm, Adam J. Kappmeyer, Joe T. Schmalz, Martin Gericke & Onesmo Balemba : Cell and Tissue Research (2013) 254 (2): 381-394

Article Summary

Obesity and type 2 diabetes are associated with autonomic neuropathy including disruption of the enteric nervous system. The authors fed a high fat diet (HFD: 72% fat) *ad libitum* to mice to assess the effects of diet and obesity on enteric neuronal number and phenotype. After 4-8 weeks of HFD, mice showed increased weight gain, insulin resistance and elevated blood glucose levels, typical features of early stage type 2 diabetes. The myenteric plexus of the duodenum was investigated for sensory, excitatory motor and inhibitory motor neuron as well as neuronal synaptic vesicles, neuronal cell death and total neuronal count. As early as 4 weeks following HFD ingestion, the number of myenteric plexus neurons was reduced and was associated with nerve damage. There was no significant difference in mean ganglionic areas, but the number of neurons per ganglionic area was reduced. HFD also induced an increase in active caspase 3 positive neurons, indicating neuronal cell death and decreased levels of synaptophysin, suggesting either impaired nerve function or a reduction in synaptic vesicles. HFD mice also exhibited axonal swelling as well as loss of neurofilaments and microtubules. The number of inhibitory motor neurons (stained with nNOS & VIP) per ganglionic area was also reduced but the number of excitatory motor neurons (ChAT & Substance P immunoreactive) and sensory neurons (Calbindin & Calcitonin-gene related peptide immunoreactive) was similar to control mice. Neuronal apoptosis of inhibitory nerve populations, neuronal swelling, damage of cytoskeletal structures and reductions in synaptophysin is hypothesized to contribute to dysmotility and other impaired GI symptoms, as seen in patients with Type 2 diabetes.

Article Commentary

Despite receiving extrinsic neural inputs via both the sympathetic and parasympathetic branches of the autonomic nervous system which modulate gastrointestinal (GI) functions, the GI tract also possesses intrinsic neural plexuses (myenteric and submucosal) that are capable of a significant degree of independent GI regulation. Disruption of the autonomic (including enteric) nervous systems, as seen in Type II diabetes, can result in dysmotility and dysregulated GI functions, which could then exacerbate appetite regulation and glycemic control. Type II diabetes is most common in the western world, where the diet is high in fat as used in the present study. While this study investigated the early disease state, continued dietary habits could exacerbate the effects observed in this study which found a loss in duodenal myenteric neurons, biased towards those of an inhibitory nature. This loss of inhibition is hypothesized to contribute to the dysmotility that results in delayed gastric emptying and reduced GI transit time observed in patients with a high BMI and poor glycemic control. The diet-induced damage observed in the enteric nervous system in the

present study is likely to affect appropriate neuromuscular control and transmission which, in turn, would be likely to affect gut-brain and brain-gut signaling via vago-vagal reflexes. Conceivably, this would lead to exacerbation of the observed gastrointestinal dysfunctions including exaggerated food intake and poor glycemic regulation. While the exact cause of HFD-induced neurodegeneration in the myenteric plexus of these mice still remains unclear, microbial factors, hyperglycemia, dyslipidemia oxidative stress and inflammatory molecules are all possible candidates for this loss.

High-fat diet and age-dependent effects on enteric glial cell populations :

Chloe Stenkamp-Strahm, Savannah Patterson, Jennifer Boren, Martin

Gericke & Onesmo Balemba : Autonomic Neuroscience (2013) 177 (2):

190-210

Article Summary

Enteric glia have been found to be involved in almost every gut function including motility, mucosal secretion and host defense. Many diseases, including Type II diabetes have shown a disruption in these supporting cells, compromising proper neuronal function. In the present study, a high fat diet (HFD: 72% fat) was fed to mice *ad libitum* for 8-20 weeks to induce Type II diabetes-like symptoms. Duodenal mucosa, submucosa and myenteric plexus tissues were used to compare glial cell expression patterns using immunohistochemistry, RT-PCR and transmission electron microscopy (TEM). Results showed that the onset of obesity began between 4 and 6 weeks with HFD mice showing higher blood glucose levels and insulin resistance. Levels of S100b (a small neurotrophin molecular secreted by enteric glial cells) were reduced in the submucosa after 20 weeks, but no changes were found in the myenteric plexus. Levels of glial fibrillary acidic protein (GFAP; intermediate filaments in enteric glial cells) did not change but levels of Sox10 (a transcription factor expressed in enteric glial cells) declined from 8 to 20 week timepoints both in control and HFD mice, showing the decline was age-dependent. At the electron microscopic level, diet or age-related structural abnormalities were not found in the myenteric plexus although there was a reduction in the area density of glial cells in the mucosa. These results suggest that obesity and Type II diabetes are associated with damage to the glial cells in the small intestine and these neuropathies may contribute to the disease.

Article Commentary

The neurodegeneration of the ENS as seen in many disease states, including Type II diabetes, may be exacerbated by dysfunction of the supporting cells of enteric neurons. Enteric glial cell function is important for maintenance of normal gastrointestinal (GI) physiology and disruption of enteroglia can result in dysmotility. The state of chronic inflammation resulting from diet-induced Type II diabetes has been shown to disrupt and dysregulate these supporting cells. The results from this study found a decrease in glial number and S100b levels in the submucosa of HFD-ingesting mice after 20 weeks although few other alterations were apparent. These results contrast other studies which have shown that there is an increase in S100b and GFAP (translating to glial cell activation and migration in the ENS) with inflammation. While the mechanisms responsible for glial cell

activation, migration and/or deletion may still be misunderstood, there is abundant supporting evidence that proper enteroglia function is crucial for enteric neuronal health and function and that these may be compromised by diet or obesity.

Conclusion

Vago-vagal neural of the GI tract clearly plays an important role in the development of obesity and it appears that all aspects of these reflexes are affected including vagal afferent neurones and fibers, central vagal efferent motoneurons as well as the enteric nervous system itself. Defining the etiology and development of obesity is clearly a difficult problem that still requires much investigation. Over a decade ago, we first postulated the idea that central vagal neurocircuits involved in GI regulation are not static relay system, incapable of modulation but, rather, are surprisingly labile and subject to a considerable degree of plasticity (Browning & Travagli, 2011; Browning & Travagli, 2001). Recent studies by other laboratory groups have also found peripheral vagal afferent neurons and fibers to exhibit similar plasticity, suggesting this may be a common mechanism of neuroregulation within vago-vagal reflexes. These studies have also highlighted the importance of experimental design and the attention to detail required in order to begin to understand the impact of diet vs feeding status vs obesity on the control, regulation and modulation of vagally-dependent reflexes.

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