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Microbe-host interactions: Influence of the gut microbiota on the enteric nervous system

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ABSTRACT

The enteric nervous system (ENS), considered a separate branch of the autonomic nervous system, is located throughout the length of the gastrointestinal (GI) tract as a series of interconnected ganglionated plexi. Given the proximity of the intestinal microbiota to the ENS, it is perhaps not surprising that the gut microbiota can influence its development and function. However, these interactions are complex and may be either direct or indirect, often involving signalling initiated by microbe-derived components, metabolites or host-derived intermediaries which subsequently affect enteric nerve excitability and GI function. Individual microbes and strains can differentially influence ENS activity and neurochemistry. In this review we will briefly summarise the role of the microbiota on ENS development, and, in some more detail, explore the mechanisms by which the microbiota can influence ENS activity and function.

1. Introduction

The gastrointestinal (GI) tract is densely innervated by a network of 200–600 million neurones which comprise the enteric nervous system (ENS). This network of neurones innervates all regions of the GI tract and is located in distinct units between either the longitudinal and circular muscle layers of the intestine or in the submucosa as ganglionated plexi; termed the myenteric plexus or submucosal plexus respectively (Furness et al., 2014). The ENS can autonomously influence the physiology and function of the GI tract, however it also communicates in a bidirectional manner with the central nervous system (CNS) by both vagal parasympathetic and sympathetic pathways, whilst vagal afferent signalling from the ENS, circular muscle layers and mucosa is facilitated by intraganglionic laminar endings, intramuscular arrays and mucosal varicose nerve endings respectively. Within the distinct plexi are discreet populations of neurones which can be classified based on their function and morphology. These include intrinsic sensory neurones, motor neurones (muscle, secretomotor and secretomotor/vasodilator) and enteric interneurons which collectively regulate key functions of the GI tract including intestinal muscle activity, gastric peristalsis and secreto- and vasomotor control (Furness et al., 2014). By virtue of its location in the gut wall, the ENS may be considered "protected" from the contents of the lumen by the epithelial barrier, mucous layer, as well as by ion and fluid secretion (Saulnier et al., 2013). These barriers, to some degree, separate the ENS from the microbiota. The most heavily colonized area of the human body is the GI tract, with bacterial concentrations ranging from 10^1 to 10^3 cells per gram in the upper intestine to 10^{11}–10^{12} per gram in the colon (Derrien and van Hylckama Vlieg, 2015; O'Hara and Shanahan, 2006). The symbiosis between host and microbiota gives rise to a collective gene system referred to as the hologenome which represents the nuclear genome, organelles, and microbiome (Bordenstein and Theis, 2015). The genetic content of the microbial communities outnumber those of the host by approximately 150-fold (Qin et al., 2010). There are multiple ways by which gut microbes can influence the host including cellular components, biosynthesis of unique molecules and dietary modification (Koppel and Balskus, 2016). Several such mechanisms have been implicated in facilitating either direct or indirect communication between the microbiota and the ENS (Fig. 1).

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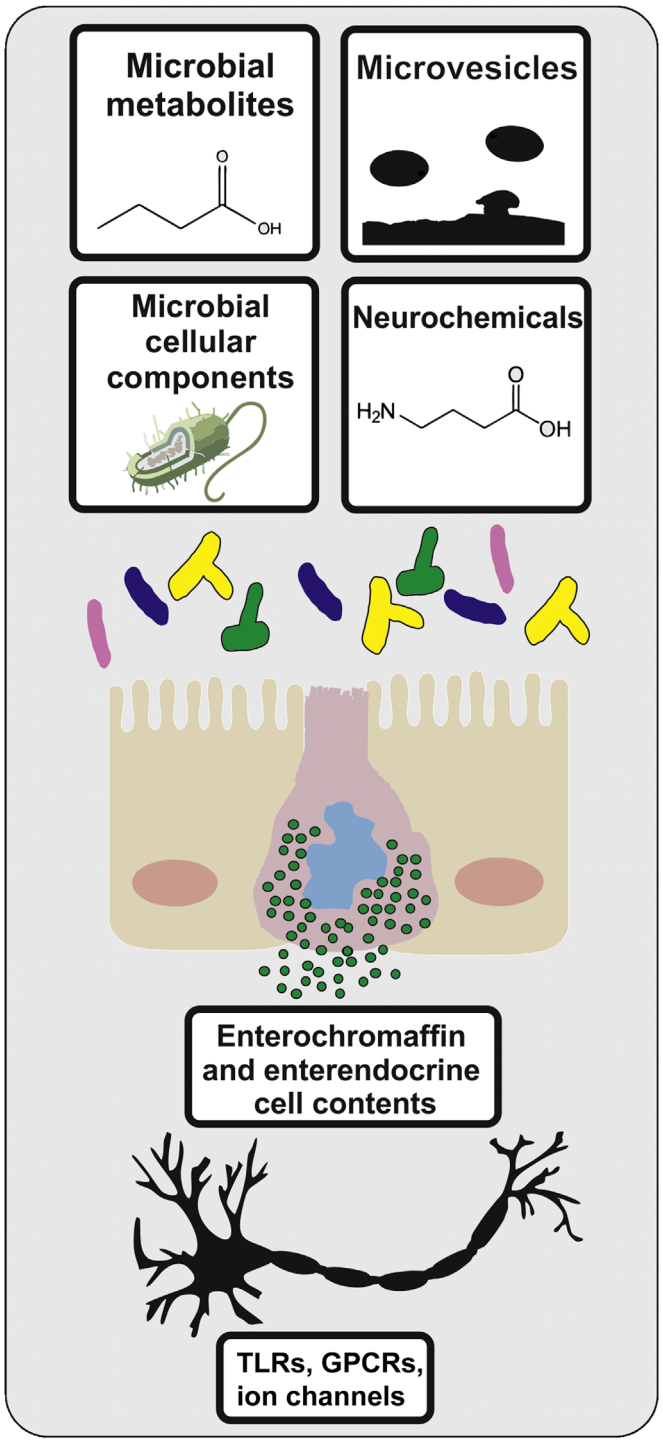

Fig. 1. Enteric neurones express Toll-like receptors, a vast array of neurochemical receptors as well as displaying sensitivity to microbial metabolites, and therefore the enteric nervous system (ENS) has the capacity to respond to microbes. Evidence suggests that the microbiota can either directly or indirectly influence the ENS through generation of microbial-derived components, neuroactive metabolites or by engaging with mucosal elements (e.g, enteroendocrine-cell derived mediators). TLR, Toll-like receptor; GPCR, G-protein-coupled receptor.

2. Impact of the microbiota on enteric neural development and plasticity

Germ free (GF) animals have proven a useful tool for investigating the contribution of microbes to host function and the impact of the microbiota on the gut-brain axis, including the ENS (Luczynski et al., 2016). The first GF animals were developed as far back as the 1800s by aseptic caesarean section and the methodology used in the generation of GF mice remains largely unchanged today (Luczynski et al., 2016). Evidence of grossly abnormal myenteric plexus architecture and size in GF rats has been reported (Dupont et al., 1965). Moreover, GF rats have been used to demonstrate the impact of the microbiota on migrating myoelectric complex (MMC) activity, though no change in ENS neurochemistry was observed following colonisation of these animals (Husebye et al., 2001). More recently, the early postnatal developmental trajectory, neurochemical profile and function of the ENS has been investigated in GF mice (Collins et al., 2014; Lomasney et al., 2014b). In the context of myenteric nerve fibre density, a GF environment significantly decreased the development of enteric neural networks in a region-specific manner on postnatal day 3 relative to both offspring born in a specific pathogen free environment or to dams colonised with a simplified microbiota (Collins et al., 2014). Nerve density was similarly decreased in the jejunum and ileum of GF mice, though preserved in the duodenum. In terms of the number of neurones per myenteric ganglia, these were decreased in both the jejunum and ileum in which increased nitrergic neurones were also observed; though this may also represent a loss of non-nitrergic neurones (Collins et al., 2014). Whilst this study did not extend analysis further into the post natal developmental period or adulthood, others have reported that the number of nitrergic neurones in the myenteric plexus of the colon and distal ileum was decreased in 4-week old GF mice without any concomitant change in cholinergic neurone number (Anitha et al., 2012). Decreased calbindin positive neurones were also noted in the jejunum of GF mice relative to specific pathogen free animals (McVey Neufeld et al., 2015). Of note, however, when GF animals were colonised the number of calbindin positive neurones not only increased in GF ganglia but also significantly increased relative to specific pathogen free animals (McVey Neufeld et al., 2015). Therefore, there is converging evidence to suggest that the microbiota can influence the development of the ENS. However, the temporal nature of this influence is unclear and some interesting questions remain. For example, why normal ENS development occurs in some regions of the small intestine and not others of GF mice; and how a simplified eight strain flora, compared to a more complex microbiota, can have similar effects on ENS development? Nonetheless, there is evidence to suggest that putative probiotics can individually influence ENS neurochemistry (di Giancamillo et al., 2010; Kamm et al., 2004). *Saccharomyces boulardii* significantly decreased the number of calbindin positive neurones, and more particularly cholinergic/calbindin positive myenteric neurones in the pig (Kamm et al., 2004). On the other hand *Pediococcus acidilactici* significantly influenced ileal neurochemistry without affecting total neuronal numbers and did not affect caecal ENS neurochemistry (di Giancamillo et al., 2010). These studies support the plasticity of the adult ENS, and, furthermore, the selective influence of particular microbes on the ENS in distinct regions of the GI tract. More recently a role for the microbiota in modulating the flow of enteric glial cells from the ENS to the mucosa has been demonstrated in antibiotic treated *Sox10::Cre;R26RConfetti* mice (Kabouridis et al., 2015). Moreover, GF mice displayed a decrease in mucosal glial cell staining relative to conventional animals which could be restored upon colonisation. However, staining of S100β was not altered in the enteric plexi of GF animals (Kabouridis et al., 2015).

3. Toll-like receptors and the enteric nervous system

Despite the separation between the microbiota and ENS, enteric neurones express pattern recognition receptors, namely