Gut Microbiota and Short Chain Fatty Acids: Influence on the Autonomic Nervous System

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Reaching across multiple fields of focus, spanning from periodontology to gastroenterology to neurobiology to behavior, interest in the influence of the microbiome in human physiology and pathology has risen over the past few decades. Microbiota co-exist in and on humans forming an evolutionarily symbiotic biological unit, a halobiont, in which disruptions in the relationship can occur through genomic alterations and mutations [1]. The human microbiome consists of bacteria, viruses, fungi, and protozoans that contribute 450 times more genes to this relationship and slightly outnumber human host cells [2, 3]. The bacteria in the gastrointestinal (GI) tract are of the most interest and exist within five phyla: *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, *Actinobacteria*, and *Verrucomicrobia*. Within the *Verrucomicrobia* an interesting bacterium has emerged, *Akkermansia muciniphila*, a mucus-degrading bacterium that influences intestinal permeability [3, 4]. The composition of individual microbiota communities depends on host lifestyle and genetics [1]. Often the *Firmicutes*-to-*Bacteroidetes* ratio is considered a method for measuring the health of a community [2–7] but has not been fully validated in other studies suggesting the measurement of phyla in feces

as a diagnostic tool may not be practical. Malfunction in the GI tract impacts other systems and leads the loss of physiological function, which disrupts the relationship between microbes and host. Gut microbial community disturbances caused by the host lifestyle (antibiotic use, food consumption, and lack of exercise), results in a decrease in diversity and have been linked to cardiovascular diseases, such as hypertension [2, 3, 5, 8], neurodegenerative Parkinson's and Alzheimer's diseases [4, 6], and even obesity [7].

Microbes in the GI tract utilize the food consumed by the host as an energy source and aid in breaking down some foodstuffs, such as fermentable fibers, resistant starches (prebiotics), and some dairy products. Through various metabolic pathways, microbes produce short-chain fatty acids (SCFAs) such as butyric acid, propionic acid, acetic acid and the alpha-hydroxy acid, lactic acid. At physiological pH, these SCFAs exist as conjugate bases; butyrate, acetate, propionate, and lactate (Fig. 1). These SCFAs, particularly butyrate is known to modulate gut functions, such as motility and permeability [3–5, 9, 10] whereas acetate and lactate are absorbed into the circulation and either used or eliminated. The response of human cells to alterations in metabolite concentrations may be regionally specific and influenced by host genomics [1, 8, 10], suggesting the pertinence to investigate microbe populations on the mucosa in various regions of the small intestine and colon. The types of receptors present on specific human cells and the numbers of receptors present alter the downstream effects in response to the presence of SCFAs in a dose-dependent manner (Fig. 1) [8–10]. Little is known about the molecular mechanisms underlying the link between microbial composition, SCFA metabolite concentrations, and overall systemic effects on host physiology. Exploring microbial community composition and associated metabolomic information in normal individuals and those with various diseases may lead to the

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Fig. 1 Physiological effects of short-chain fatty acids (SCFAs) produced by gut microbes on various organs. SCFAs produced by gut bacterial either bind to intestinal endothelial receptors activating various second messenger pathways, pass through cell membranes to influence gene expression, or move through endothelial cells into the portal system (1) to bind to receptors elsewhere. Binding to GPCR 41 receptors in blood vessels induces vasodilation; binding to Olf78 receptors in the blood vessel induces vasoconstriction (2) and renin secretion in the kidney (3); both increase arterial blood pressure

(ABP). Once in the bloodstream, SCFAs can cross the blood brain barrier *via* monocarboxylate transporter (MCTs) (4) and influence various neurons with different receptor expression causing cascading effects, such as increasing sympathetic outflow to increase heart rate (5) and vasoconstriction or by increasing parasympathetic outflow to alter gut motility and the microbes themselves (6). As well as traveling through the blood to the brain, SCFAs can increase the retrograde communication between the gut and the brain, causing downstream effects (7).

development of recognizable biomarkers (host or microbial byproducts) that indicate the possibility of pathology, which hopefully will aid in preventative therapeutic interventions. Understanding the specific receptors to which SCFAs bind and their downstream effects could lead to therapies targeting specific receptors and thus restore gene expression and changes in neuronal signaling. In this insight, we seek to investigate the role of SCFAs produced by resident gut microbes, in regulating the autonomic nervous system and the implications of this regulator role in the development of human pathology.

SCFA Modulation in the Vagus Nerve

Various intraluminal and intramucosal factors can modulate enteric neuronal function, potentially increasing neuronal excitability, which, if occurring over long periods of time alters neuronal morphology, gene expression, and excitability [9, 10]. Most SCFAs present in the plasma are byproducts of bacterial metabolism and act as physiological modulators of the enteric nervous system [8, 9]. The presence of lipids in

the intestines stimulates vagal afferent pathways [9, 10], possibly altering expression in specific brain regions, and increasing parasympathetic output from various brain areas, altering the autonomic influence on the body. SCFAs for example butyrate activate intestinal vagal nerves by directly acting on the terminals in a cholecystinin-independent manner [9], indicating other mechanisms of neurotransmitter release that are currently unknown. In contrast to long-chain fatty acids, SCFAs show a quicker and longer-lasting afferent chemical response, possibly promoting the changes in gene expression. Feeding Sprague–Dawley rats a resistant starch diet increases GI luminal SCFA concentrations and increases acetylcholine synthesis, monocarboxylate transporter 2 receptor expression, neuronal excitability, and histone acetylation [10], displaying the impact microbe SCFAs have on host gene expression. Administration of butyrate increases the proportion of choline acetyltransferase in myenteric neurons, promoting GI motility and contractility [10]. Understanding the underlying mechanisms by which butyrate increases the activity of cholinergic neurons and enhances gut motility could be potential therapeutic targets for individuals suffering from GI dysfunction.

In addition, insulin-resistant rats fed a high-fat diet have increased whole-body acetate turnover and increased fecal and plasma acetate concentrations both of which are accompanied by a rise in parasympathetic activation [7]. The study conducted by Perry and colleagues revealed an acetate-induced increase of parasympathetic vagal stimulation, acutely driving glucose-stimulated insulin secretion through the autonomic activation of pancreatic β -cells. This condition of hyperinsulinemia leads to a fivefold increase in gastrin and a threefold increase in ghrelin, which in turn leads to a dramatic intake of calories resulting in obesity [7]. Understanding the underlying mechanisms by which acetate concentrations influence the autonomic parasympathetic outflow could lead to therapeutics for treating obesity and diabetes. Even though the animal models vary and the downstream effects of increased SCFAs production differ, an increase in SCFA production by gut microbes increases parasympathetic output through the vagus and glossopharyngeal nerves to impact host function. The difference in effects may be attributed to the various transporters/receptors with which SCFAs interact: monocarboxylate transporter (MCTs), G protein-coupled receptors (GPCRs), or olfactory receptors [8, 10]. The expression and localization of these receptors found in specific neurons and/or various regions of the body, or perhaps their concentrations may influence the receptor response and phenotypic output [8, 10–12]. Comprehension of the downstream effects of SCFA-receptor activation and the influence on the autonomic nervous system will lead to novel discoveries in pathophysiology and lead to new approaches in therapeutics and medicine.

SCFA Modulation in the Sympathetic Nervous System

Disruption of the microbiome in disease models in combination with a decrease in intestinal barrier integrity may lead to alterations in SCFA concentrations in the blood. The increase in permeability allows a rise in SCFA concentrations in the blood, leading to enhanced activation of sympathetic nerves by SCFAs [5, 11]. A gain in sympathetic outflow modulates various organ systems, including those of the cardiovascular system controlling heart rate, contractility, vasoconstriction, and renin secretion. Changes in any of these, result in changes in arterial blood pressure (ABP). Interestingly, pre-hypertensive young spontaneous hypertensive rats (SHRs) exhibit a decrease in tight-junction protein expression, which is preceded by an increase in permeability, sympathetic output, and a shift in the gut microbial composition [3, 5]. Measuring individuals assumed at risk of developing hypertension for tight-junction protein gene expression could be used as a biomarker to

determine whether a person is at increased risk of intestinal permeability pathology.

Increasing permeability of the GI allows for higher concentrations of signal molecules, such as SCFAs and cytokines, to reach and cross the blood-brain barrier [8], targeting various regions of the brain and resulting in effects dependent on receptor expression and localization. In SHRs, a shift in the SCFA concentrations stimulates the paraventricular nucleus (PVN) in the hypothalamus, which increases sympathetic signaling to the GI [3, 5]. An increase in autonomic outflow to the GI alters the environment of the GI causing a shift in microbial composition, Yang *et al.* [3] reported a shift to an increase in lactic acid-producing *Firmicutes*. This rise in lactic acid production acts in a positive feedback loop by further increasing PVN sympathetic output, possibly propagating continuous shifts in microbial populations and potentially leading to hypertension.

Microbial dysbiosis is a state in which there is a maladaptation or imbalance in microbial composition. Dysbiosis may induce a shift promoting abnormally high numbers of acetate-producing bacteria. Acetate is the end-product of ethanol/ alcohol metabolism and has a structural homology to glutamate carboxyl terminals [11] with the ability to act in the brain through various mechanisms. When acetate activates N-methyl-D-aspartate receptors (NMDARs) in the central nucleus of the amygdala, sympathetic activity increases resulting in a rise in ABP [11]. In a dose-dependent manner acetate has been shown to induce excitotoxicity in dopaminergic PC12 cells [12] by either binding to NMDARs or entering the cell presumably through MCTs. The opening of NMDARs channels increases cytosolic Ca^{2+} concentrations acting on several second messenger systems. The transport of acetic acid into the cell through MCTs decreases the pH, altering cell functions. These actions point towards upregulation and expression of the pro-inflammatory cytokine tumor necrosis factor alpha and contribute to cell death [12] and possibly contributing to neurodegenerative diseases such as Parkinson's and Alzheimer's. Understanding the pathways through which different SCFAs induce changes in brain signaling and linking the changes in autonomic outflow to adaptations in the microbial population could potentially lead to decreases in sympathetic and parasympathetic output through dietary changes that alter the diversity the gut microbiome selecting for increases or decreases in specific populations.

Influence of SCFAs on Hypertension

The influence of microbial metabolites on hypertension and other cardiopulmonary pathologies is a relatively new focus of study with much still to be discovered. Unraveling the underlying mechanisms by which microbiota secretions

and metabolites act on human cells will impact human health and potentially lead to new methodologies of treatment. According to Koch's third postulate, if microorganisms contribute to disease, then transferring the microorganism to a healthy organism will induce pathogenicity. Using the third postulate as a guide, when normotensive Wistar-Kyoto (WKY) rats receive a fecal microbiota transplant (FMT) from a stroke-prone (SHR), there was an increase in ABP [2]. Along with the rise in ABP, the recipient rats had an increase in SCFA concentrations in the fecal matter accompanied by a shift in microbiome community composition [2], mimicking the diseased microbial configuration.

Microbial composition shifts alter the production of metabolites produced by various bacteria, potentially influencing several systems in the human body. Previous reports using mice, demonstrated that acetate and propionate bind to various GPCRs acting as chemical sensors in the circulatory system and the kidney to play roles in renin secretion and blood flow resistance [8]. Olfactory (Olf) 78 receptors are localized in a subset of smooth muscle cells composing renal artery branches and small resistance vessels associated with the renal juxtaglomerular apparatus, ideal locations for influencing renin secretion and blood flow resistance. Propionate binding to Olf78 receptors induces the release of renin from juxtaglomerular cells in the afferent arterioles, resulting in an increase in ABP. However, if propionate binds to GPCR 41, the ABP drops [1, 8]. Thus, these two receptors are capable of counteracting each other to maintain homeostatic mean arterial pressure in response to varying SCFA concentrations [8]. Building knowledge of the various receptors to which SCFAs bind in different tissues could lead to potential antagonist and agonist drug development to aid in the regulation of ABP.

WKY rats have diverse microbial populations from the major five phyla discussed earlier [2, 3] and when the diversity of the ecosystem decreases it becomes unstable and susceptible to perturbation. Yang *et al.* found that the SCFA concentrations in fecal samples differed depending on the overall microbial composition [3]. SHRs had significantly higher lactate levels associated with diminished acetate and butyrate concentrations than WKY rats. WKY rats that receive a FMT from stroke-prone SHRs show an increase in fecal SCFA acetate, propionate, and butyrate concentrations, which increase above those in WKY and stroke-prone SHRs do not receive FMT [2]. The difference in SCFA concentrations between WKY and SHRs and the shift in SCFA concentrations following FMT of disrupted microbiota into WKY rats are correlated with an increase in *Firmicutes* and decreases in *Bacteroidetes*, *Proteobacteria*, and *Verrucomicrobia* species [2, 3, 5]. It appears that the initial onset of microbial disruption from a

normal composition causes an increase in SCFA production [2, 3, 5], inducing an elevated ABP though an increase in sympathetic outflow. Alterations in microbial communities can modify intestinal permeability, possibly allowing elevated amounts of SCFAs to enter the blood and inducing various physiological responses. An exploration into the mechanisms promoting the growth of various species following initial disruption could lead to potential probiotic and prebiotic therapies to reestablish diversity in the microbial community and restore the balance among species. Several groups have demonstrated a shift in SCFA concentrations and microbial composition in fecal samples [2–7], but there is still a need for exploration into the composition of bacterial populations associated with the mucosal layer throughout the GI. Characterization of various bacterial populations at different sites in the GI may lead to novel discoveries on how the metabolites of bacterial populations influence host cell function.

Influence of SCFAs on Neurodegeneration

Emerging evidence has linked microbial dysbiosis and changes in metabolite production in the GI to neurodegeneration [4, 6]. Several neurodegenerative disorders, such as Parkinson's and Alzheimer's, are associated with the aggregation of proteins in specific regions of the brain, impacting function inducing pathogenicity [4, 6]. Interestingly, aggregation of the protein alpha-synuclein (α Syn) is first seen in the GI of Parkinson's patients [4], suggesting that the pathogenicity spreads from the GI to the CNS through the vagal and glossopharyngeal nerves.

The use of animal models allows researchers to establish various pathways that stimulate the overexpression or aggregation of specific proteins in various brain regions. Fecal samples from individuals with Parkinson's disease (PD) have a lower acetate concentration than those taken from age-matched controls [4]. The administration of Parkinson's patient samples into germ-free mice results in a similar decrease in the relative abundance of acetate in fecal samples [6]. This decrease in acetate could indicate compromised intestinal integrity, allowing more acetate to move from the lumen into the bloodstream to be distributed throughout the body. Exploration of the possible effects of an increase in acetate concentrations in various tissues could lead to discoveries of downstream mechanisms inducing pathogenicity.

Most SCFAs have the capacity to cross the blood-brain barrier via the MCT and affects the central nervous system [6, 7, 10]. SCFA administration in the Thy1- α Syn (alpha-synuclein-overexpressing, ASO) mouse induces an increase in microglia activation in a region-specific manner [6]. Microglia activation is associated with the upregulation

of α Syn aggregation. Microglia in germ-free mice are unable to complete morphological changes in order to induce an inflammatory response [6], showing a correlation between gut microflora secretions and the maintenance of neuronal and glial health. Transplantation of PD fecal samples into ASO mice increases motor impairment [6], suggesting that microbiota contribute to synucleinopathies and progression of the neurodegenerative disorder.

Dysbiosis of bacterial phyla is seen in the GI of individuals with PD in comparison to control samples [4, 6]. Patients with PD display a decrease in *Bacteroidetes* and *Firmicutes* accompanied by an increase in *Proteobacteria*, *Actinobacteria*, and *Verrucomicrobia* species *Akkermansia muciniphila* [4, 6]. Bacterial disruption is associated with several pathologies spanning several systems of the human host however, specific community disruptions and underlying mechanisms of the changes in microbial metabolite production and pathogenicity is still largely unknown.

Conclusions

Over the past two decades, it has been made clear that the composition of the gut microbiome impacts various physiological functions and plays a major role in the development of pathology. Often the *Firmicutes*-to-*Bacteroidetes* ratio is used as a diagnostic tool, but the literature is controversial [2–7] illuminating its insufficiency for clinical use at the current level of understanding. Potential direction of investigation into not only the bacterial composition of fecal matter but also the composition of bacterial populations associated with the mucosal cells of the host could present a more reliable and accurate assessment of microbial communities associated with pathology and health. The varying compositions of microbial communities between pathologies implies a need for future research into microbial diversity in disease states compared to healthy and possible high-risk individuals.

Dysbiosis results in a shift in metabolite production and the concentrations found in the GI, blood plasma, and brain regions. Responses to various SCFAs concentrations may differ regionally [9], depending on receptor expression on resident cells [1, 8], possibly influenced by host genomics [10]. Pathogenicity can change receptor responses to SCFA binding [10]; in normal physiology, binding may cause a reaction that completely opposes the activity induced in a pathogenic state. Investigation into specific regional receptor responses to SCFA binding in normal and pathogenic models will lead to novel discoveries underlying

mechanisms propagating various pathologies, such as hypertension and Parkinson's disease. Distinguishing early biomarkers, tight-junction protein expression and gut microbiota diversity, will aid in the development of therapies targeting prevention of the early onset of cardiovascular diseases and neurodegeneration.

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Conflict of interest The authors declare that they have no conflict of interest.

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