

Review article

Butyrate, neuroepigenetics and the gut microbiome: Can a high fiber diet improve brain health?



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HIGHLIGHTS

- Interest in how diet influences brain function via the gut microbiome is growing.
- Butyrate can protect the brain and enhance plasticity in neurological disease models.
- Gut microbiota produce butyrate by fermenting carbohydrates in a high fiber diet.
- Hypothesis: A high fiber diet can elevate butyrate to prevent/treat brain disorders.

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ABSTRACT

As interest in the gut microbiome has grown in recent years, attention has turned to the impact of our diet on our brain. The benefits of a high fiber diet in the colon have been well documented in epidemiological studies, but its potential impact on the brain has largely been understudied. Here, we will review evidence that butyrate, a short-chain fatty acid (SCFA) produced by bacterial fermentation of fiber in the colon, can improve brain health. Butyrate has been extensively studied as a histone deacetylase (HDAC) inhibitor but also functions as a ligand for a subset of G protein-coupled receptors and as an energy metabolite. These diverse modes of action make it well suited for solving the wide array of imbalances frequently encountered in neurological disorders. In this review, we will integrate evidence from the disparate fields of gastroenterology and neuroscience to hypothesize that the metabolism of a high fiber diet in the gut can alter gene expression in the brain to prevent neurodegeneration and promote regeneration.

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1. Introduction

The relationship between our gut microbiota and nervous system is a large part of the gut-brain axis that has attracted increasing interest in recent years. It is estimated that 90% of the cells in the human body are of microbial origin, and the vast majority of these microbiota are comprised of 15,000–36,000 species of commensal and symbiotic bacteria that reside within the lumen of the gut [1,2]. A diverse microbial community is crucial for our health and disease prevention based on microbiome studies (*i.e.*, metagenomic sequence analyses) and perturbed energy homeostasis that has been observed in germ free mice [3]. Although it is not yet clear how gut microbiota positively and negatively affect brain function, multiple mechanisms are likely to be involved. Gut bacteria, have a prodigious metabolic capacity and some microbe-derived metabolites enter the circulation and can cross the blood-brain barrier. There is growing evidence that these microbes produce neurotransmitters, such as GABA and serotonin, modulate the immune system, alter epigenetic markers and produce bioactive food components and energy metabolites [2,4,5]. Thus, dietary manipulation to achieve a symbiosis that can improve the health of the microbiome and our brains is an attractive idea currently under investigation.

In this review, we will focus on the short chain fatty acid (SCFA), butyrate, which is most commonly produced by bacteria in the colon, and its role as a potential therapeutic for neurological diseases. Butyrate is an attractive therapeutic molecule because of its wide array of biological functions, such as its ability to serve as a histone deacetylase (HDAC) inhibitor, an energy metabolite to produce ATP and a G protein-coupled receptor (GPCR) activator. Pharmacologically, butyrate has had a profoundly beneficial effect on brain disorders ranging from neurodegenerative diseases to psychological disorders. In this review, we will discuss how butyrate is made and the pharmacological effects of butyrate in neurological disorders. Finally, we will summarize the current evidence that high fiber, butyrate-producing diets are capable of improving the health of our brains.

1.1. Sources of butyrate

Butyrate is synthesized via the fermentation of otherwise non-digestible fiber by bacteria in the colon. Two acetyl CoA molecules are condensed into acetoacetyl CoA, which is converted through L(+)-beta-hydroxybutyryl CoA and crotonyl CoA intermediates to butyryl CoA. Butyryl CoA is then converted to butyrate either by butyrate kinase or butyryl CoA and acetate CoA transferase (as shown in Fig. 1) [6,7].

Bacteria, such as those from the *Clostridium*, *Eubacterium*, and *Butyrivibrio* genera, are able produce butyrate in the gut lumen at mM levels [7,8]. In addition to producing butyrate as an endpoint, bacteria produce fermentation intermediates, including lactate, succinate or formate, which are used by the bacteria themselves to proliferate and survive [9,10]. Butyrate is also utilized by microbiota and serves as the primary energy source of colonocytes (as discussed below), making this a vital and mutually beneficial relationship. High fiber foods, summarized in Table 1, that enable these butyrate-producing bacteria to thrive include resistant starches (*e.g.*, whole grain and legumes) and fructo-oligosaccharides (FOS) (*e.g.*, bananas, onions, and asparagus). In fact, within two weeks of a high FOS diet, rats showed increase butyrate in the large intestine without changing the total number of anaerobic bacteria [11]. Similar results were seen in another study with diets containing FOS as well as resistant starches, but were not observed in the starch free wheat bran diet, which produces less butyrate [12]. This study, as well as many others, demonstrates that different sources of fiber

Table 1

Summary of the proportions of SCFA produced with different forms of carbohydrates. Adapted from Smith et al. [102].

Carbohydrate	Proportion of SCFA energy (kJ) produced		
	Butyric acid	Propionic acid	Acetic acid
Cellulose	0.33	0.24	0.43
Gum arabic	0.17	0.28	0.56
Lactulose	0.36	0.16	0.48
Oat bran	0.38	0.24	0.38
Pectic substances	0.32	0.17	0.51
Resistant starch	0.55	0.21	0.24
Wheatbran	0.34	0.23	0.42
Xylan	0.06	0.23	0.71

yield different levels of butyrate so care must be used in selecting the appropriate fiber diets to increase butyrate levels.

Aside from being produced by bacterial fermentation, butyrate can also be produced in much lower concentrations by mammalian cells through fatty acid oxidation and glucose metabolism [13,14] and can be found in plant oils and animal fats [4]. Butyric acid (the acid form of butyrate) is also present in the milk of ruminant animals, such as cows. Butter contains 3–4% butyric acid, in the form of tributyrin (butyryl triglyceride), making it the richest dietary source of butyrate [15]. Interestingly, the term butyrate originates from the Greek word for butter [16,17]. One molecule of tributyrin is metabolized into three butyrate molecules by intestinal enzymes. Tributyrin (1 g/kg) was able to elevate portal vein concentrations of butyrate to 2.4 mM after 1 h in rats [18].

1.2. The functions of butyrate

1.2.1. Histone deacetylase inhibitor

Histone acetylation is a post-translational modification by an epigenetic protein, which are proteins that bind to chromatin and influence chromatin structure to change the propensity that a gene is transcribed or repressed. Acetylated histones cause the chromatin structure to loosen by weakening electrostatic attraction between the histone proteins and the DNA backbone. This process enables transcription factors and the basal transcriptional machinery to bind and increases transcription. Acetyl groups are added to highly conserved N-terminal L-lysine residues by histone acetyltransferases (HATs) and removed by histone deacetylases (HDACs). Reduced HAT activity, lower global histone acetylation and transcriptional dysfunction are characteristics of many neurodegenerative diseases [19]. Thus, HDAC inhibitors have become attractive therapeutic candidates due to their ability to increase

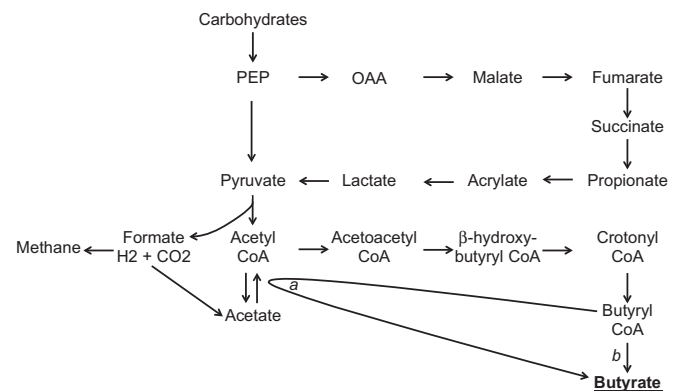


Fig. 1. Schematic representation of the carbohydrate fermentation pathways that lead to butyrate production in the large intestine. The final enzymes involved in the formation of butyrate are: (a) Butyryl CoA:acetate CoA transferase (b) Phosphotransbutyrylase/butyrate kinase. Adapted from Pryde et al. [7].

histone acetylation and promote the expression of pro-survival, pro-regenerative and pro-plasticity genes.

Sodium butyrate (NaB), the sodium salt form of butyrate commonly used in pharmacological studies, is a well-known HDAC inhibitor that results in increased histone acetylation when applied to cells in culture in the high micromolar range [20,21]. Studies from our own lab demonstrated that NaB treatment could resist oxidative stress *in vitro* and *in vivo* [22–25]. These salutary effects were highly correlated with HDAC inhibition as a mechanism of protection. In multiple models of Huntington's disease, we have shown that NaB and phenylbutyrate, a structurally similar analog, rescues histone acetylation, prevents neuronal cell death and extends the lifespan of mice [23,26].

Numerous subsequent studies have shown that NaB's salutary effects span many neurological disease models and aspects of the pathophysiology of disease. For example, NaB can protect neurons from cell death in models of Parkinson's disease [27–29] and cisplatin-induced hearing loss [30], where NaB was able to reverse the disease-associated reduction in histone acetylation. Similarly, NaB was able to reduce the infarct size in models of ischemic stroke, limiting the damage to the brain and improving behavioral outcomes [24,31–33]. *In vitro* and *in vivo* (via intraperitoneal injection) data from our own laboratory also suggests that butyrate can induce resistance to oxidative stress and increase histone acetylation and enhance gene expression of a number of genes in the high micromolar range [24,34]. Altogether, these observations are consistent with the idea that NaB can modulate the expression of a large number of genes to affect numerous pathophysiological pathways. The prospect of accomplishing this goal with a single, naturally occurring small molecule is exciting.

NaB has also demonstrated a profound effect on improving learning and memory, particularly in cases of disease-associated or toxicity-induced dementia. In mouse models of Alzheimer's disease, histone acetylation is restored and expression of learning-associated genes is increased with NaB treatment [35,36]. While NaB had no effect on the contextual memory on wild-type mice, contextual memory in the transgenic mouse model showed significant improvements, even at late stages of Alzheimer's disease. These improvements in learning and memory have also been demonstrated in models of memory impairment from a toxic overload of metals [37,38], traumatic brain injury [39] or neurological infections [40,41].

As HDAC inhibitors influence the transcription of numerous genes, it seems unlikely that a single gene is responsible for its neurotrophic effects. However, many studies have shown that at least some of these beneficial effects can be attributed NaB's ability to increase acetylation around the promoters of neurotrophic factors, such as BDNF, GDNF and NGF and thus increasing their transcription [41–48]. Other studies have demonstrated the importance of immediate early genes, including c-Fos and Homer1a, which are activity dependent genes involved in plasticity [44,49–52]. Based on these data, NaB is capable of upregulating a suite of genes that promote survival, plasticity and regeneration.

It should also be noted that the effects of HDAC inhibitors are not specific to histone proteins alone. In fact, a growing list of over 1700 proteins can be acetylated on their lysine residues, and HDAC inhibitors block their deacetylation as well [53]. Thus, the effect of HDAC inhibitors on non-histone proteins should not be overlooked. Acetylation plays an important role that affects the enzymatic and metabolic activity of many proteins. For example, our group has shown the transcription factor Sp1 is acetylated and activated by oxidative stress, and butyrate, as well as other HDAC inhibitors, can enhance this protective adaptive response to promote cell survival [22,23,54].

1.2.2. Metabolism and mitochondria

Butyrate plays two major roles in metabolism and mitochondrial activity. First, butyrate can serve as an energy substrate. In fact, colonocytes have adapted to use butyrate as their primary source of energy, which accounts for approximately 70% of ATP produced [55]. Given overwhelming use of butyrate as an energy substrate in the colon and the microbiome, much of the research to date has focused on its metabolic effects in the colonic epithelium. It was recently shown that germ free mice, which lack a microbiome, had a significantly reduced NADH/NAD⁺ ratio and reduced ATP levels in the colon compared to conventionally raised mice with a normal microbiome [56]. Butyrate was able to rescue the diminished mitochondrial respiration in the germ free mice, which demonstrates its importance in energy homeostasis. It was also found that cancerous colonocytes undergoing the Warburg effect, defined by their preferential use of glycolysis rather than mitochondrial respiration, allows butyrate to accumulate in the nucleus where it can act as an HDAC inhibitor [57]. However, in normal colonocytes the cells rapidly utilize butyrate for energy via mitochondrial β -oxidation. Interestingly, mitochondrial metabolism of butyrate does produce more acetyl-CoA, which can be used by HATs to add acetyl groups to proteins so under either condition butyrate could in theory increase acetylation and affect gene transcription.

While the metabolic events in the colon may appear disconnected from that of the brain, it's important to consider the immense energy demands of the brain and the energy dyshomeostasis that occurs in the brain in many neurological diseases. Perhaps the best cited example is the reduced glucose utilization in the Alzheimer's brain, which occurs at the earliest stages of the disease and well before memory loss [58,59]. This reduction in glucose utilization has prompted a number of studies to identify therapeutic strategies to provide alternative sources of energy to fulfill the brain's energy requirements. For example, the use of caprylic triglycerides as a medical food to enhance ketogenesis via increasing β -hydroxybutyrate has been shown to improve cognitive scores in Apo-E4 negative Alzheimer's disease patients after 45 days [60]. Longer time points were less successful, but it is likely that these treatments would need to begin before significant cognitive symptoms to achieve maximum effectiveness. Currently no studies have examined the metabolic effect of gut-derived butyrate on the brain. However, we hypothesize that if sufficient butyrate levels could be reached in the brain, butyrate could stand in as an energy substrate, as in the colon, and restore energy homeostasis, but the precise concentration to affect brain physiology via changes in the diet, gut microbiome or through pharmacological supplementation remain undefined.

Reduced brain glucose availability is believed to contribute to mitochondrial dysfunction in acute and chronic neurological diseases but could, in theory, be aided by the presence of butyrate's direct and indirect effects on energy metabolism. In this context, butyrate can: (1) directly affect energy metabolism by acting as a substrate for beta-oxidation; (2) can upregulate genes involved in mitochondrial biogenesis (e.g., PGC1 α) via its effects as a selective HDAC inhibitor; and (3) via its ability to affect the acetylation of a wide number of metabolic proteins. This last effect of butyrate and other HDAC inhibitors on metabolism was brought to light by the studies demonstrating that nearly every enzyme involved in glycolysis, gluconeogenesis, the TCA cycle, fatty acid metabolism and glycogen metabolism is acetylated [61]. The potent HDAC inhibitor, TSA, was able to significantly increase acetylation of these proteins. In the case of malate dehydrogenase, acetylation was increased by both glucose and TSA to lead to increased activity levels. To a large degree, these studies have been replicated with NaB by the lab of João Quevedo, which showed that NaB can restore Complex I–IV, as well as the TCA cycle activity, after

being repressed by amphetamines and ouabain in animal models of mania [62–64]. These studies demonstrate that butyrate is also capable of increasing mitochondrial activity, which can help to rectify the disease-associated mitochondrial dysfunction in the brain.

1.2.3. Protein-coupled receptor activator

G protein-coupled receptors (GPCR) are the largest and most diverse family of transmembrane proteins. They are comprised of 7 transmembrane α -helices, which bind extracellular signals, such as light-sensitive compounds, hormones, growth factors and neurotransmitters, and activate signal transduction pathways inside of the cell, primarily the cAMP and phosphatidylinositol signaling pathways [65]. With the role in detecting extracellular signals, GPCRs are critical for a number of physiological functions including regulation of immune system, autonomic nervous system regulation, sensory (taste and smell) function, and maintaining energy homeostasis. Dysfunction of GPCRs is associated with a number of diseases, which has made them the target for more than 40% of prescribed drugs [4].

In 2003, two previously orphan GPCRs, with no known ligands (GPR41 and GPR43), were identified as receptor targets for SCFA and aptly renamed free fatty acid receptors (FFAR) 3 and 2, respectively. Although GPR41 and GPR43 are related showing 52% sequence similarity and identified as tandemly encoded genes in chromosome 19, they differ on their preference in chain length of the SCFA ligands. FFAR2 is more specific to the shorter aliphatic chains of acetate and propionate, while FFAR3 preferentially binds propionate, butyrate and valerate [66].

FFAR3 appears to play a significant role in balancing energy metabolism through intestinal gluconeogenesis (IGN) and the sympathetic nervous system [67]. FFAR3 is highly expressed in the sympathetic nervous system and knockout of FFAR3 in mice show reduced sympathetic ganglion activity. What is most intriguing is that while it has been demonstrated that SCFA (propionate) activate the ganglia activity via FFAR3, the ketone body, β -hydroxybutyrate, which is produced under conditions of starvation or ketosis but structurally similar to butyrate, inhibits ganglia activity by functioning as an antagonist to FFAR3. Although both butyrate and propionate are agonists of FFAR3 in the nervous system, only propionate uptake in the IGN is dependent on FFAR3. This finding demonstrates a novel interaction between metabolic substrates and sympathetic nervous system activity [68].

Butyrate also signals through GPR109a, which is widely expressed in the in colonocytes and T cells, but has also been found in microglia [69–72]. The expression of GPR109a is reduced in human colon cancer cells, and the forced expression of GPR109a in these cancer cells induces apoptosis [69]. While it requires millimolar concentrations of butyrate to activate these receptors, the concentration of butyrate in the colon is more than sufficient to enhance activity the GPR109a. This has the potentially protect the healthy colon tissue through its anti-inflammatory signaling. Indeed, butyrate was shown to suppress colonic inflammation by inducing apoptosis in T cells residing in the colon [70]. Interestingly, knocking down GPR109a (or its transporter Slc5a8) in these cells increased the inflammatory response. More recently, it was shown that GPR109a is upregulated in the substantia nigra in Parkinson's disease patients, where immunostaining for GPR109a was colocalized with microglia [71]. Treatment with β -hydroxybutyrate induced anti-inflammatory effects in both *in vitro* and *in vivo* models of Parkinson's disease through GPR109a activation and down regulating NF- κ B activation [72]. The neurons were also protected from LPS-induced injury and improved behavioral outcomes in the animal models. In contrast to the study above, where β -hydroxybutyrate was shown to be an FFAR3 antagonist, here β -hydroxybutyrate is acting as a GPR109a agonist. However, the

effect of β -hydroxybutyrate with respect to GPCRs appears to be under significant debate, as other groups have identified β -hydroxybutyrate as a FFAR3 agonist [73]. However, the Parkinson's disease studies demonstrate that the activation of GPR109a leads to anti-inflammatory effects in the brain and suggest that it is a good target for therapeutics in PD.

1.3. High fiber diets and the brain

High fiber diets have numerous reported health benefits in reducing risk of type 2 diabetes, colon cancer, obesity, stroke and cardiovascular disease, making it a widely recommended healthy diet. Many of the reported effects have been associated with the microbiome and its ability to produce SCFA, like butyrate. Much of the butyrate produced in the colon is used as an energy source by the colonocytes, but some butyrate can also exit the colon through the portal vein, where the liver absorbs another large portion [74,75]. However, the distal colon is not connected to the portal vein, allowing for some systemic butyrate to be circulated. Indeed, there are many reports of high fiber diets increasing blood levels of circulating butyrate [75–77]. These later reports raise the possibility that increases in circulating butyrate could affect CNS function directly.

To date, only a handful of studies have probed the mechanistic basis surrounding the beneficial neurological effects of the high fiber diet and butyrate. A recent study demonstrated that germ free mice have increased blood brain barrier (BBB) permeability, when compared with specific pathogen free mice containing a healthy microbiota [78]. The increased permeability was associated with decreased levels of tight junction proteins, claudin 5 and occludin in the frontal cortex, hippocampus and striatum. Colonizing the germ free mice with the butyrate-producing bacteria, *Clostridium tyrobutyricum*, or an oral gavage (for 3 days) of NaB restored BBB permeability to the healthy levels of the pathogen free mice, while simultaneously increasing brain histone acetylation and expression of occludin and claudin 5. This study demonstrated the strong and important connection between the microbiota, butyrate and the brain. Another study found significant immune benefits in the brain of mice fed a diet high in fermentable (soluble) fiber and found that they recovered faster from endotoxin-induced sickness [79]. Not only did the diet successfully increase all SCFAs in the colon, but they also found significant benefits by attenuating neuroinflammation resulting from the diet. Mice fed the soluble fiber diet showed an increase in IL-1RA, a cytokine and inhibitor of the pro-inflammatory, IL-1 β , in the brain after exposure to the endotoxin, lipopolysaccharide (LPS) and a decrease in IL-1 β and TNF- α . IL-4 is a cytokine shown to increase IL-1RA and as expected IL-4 mRNA was also increased in the brains of mice on the soluble fiber diet after LPS treatment. IL-4 expression is enhanced by increased histone acetylation. Thus, the authors hypothesized that the elevated butyrate from the dietary fiber fermentation may contribute to the immune response. In contrast to the soluble fiber diets, splenocytes from mice on the insoluble fiber diet did not increase IL-4 production when exposed to butyrate *in vitro*.

1.4. The microbiome and cognition

Several studies have examined the beneficial effects of a high fiber diet on memory and cognition. In these studies the diet and/or microbiome are manipulated to enhance brain function. For example, children on a high fiber diet demonstrate better cognitive control (e.g., multitasking, working memory and maintaining focus) than children who typically ate a lower fiber diet [80]. Other studies have examined the effects of probiotics that would increase butyrate-producing bacteria. These studies showed that the probiotics reduced anxiety in rats and lowered psychological stress

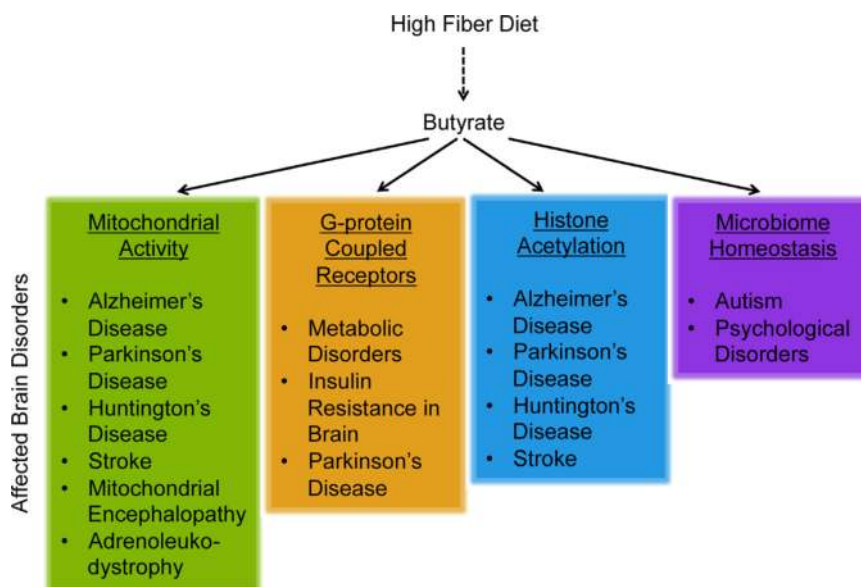


Fig. 2. The proposed mechanisms for the neuroprotective effects of butyrate and the diseases which may benefit from butyrate treatment or a high fiber diet.

in human subjects [81]. A similar study in subjects with chronic fatigue syndrome showed reduced anxiety, a common symptom of the disease, with the use of probiotics [82]. Another study provided healthy subjects with a fermented milk product and used fMRI to assess changes in the brain [83]. However, no significant difference was observed in fecal microbiota samples in patients who received the fermented milk product.

Perhaps one of the most interesting brain-microbiome connections lies in autism, where an overwhelming 70% of autistic children suffer from gastrointestinal (GI) symptoms [84]. The degree of GI symptoms, most commonly diarrhea and bloating, is often positively correlated with the severity of autism. This correlation has launched numerous studies to determine whether the microbiome of autistic patients differ from those without autism. Indeed these studies have found evidence of decreased *Bifidobacteria* and *Prevotella* and higher levels of *Lactobacillus*, *Sutterella* and *Firmicutes* based on cultures from fecal samples, and these differences appear to be independent of diet [84–87]. Some studies have even reported an alleviation of autism symptoms in children with late-onset autism with the use of vancomycin, a poorly absorbed antibiotic; though the effects quickly diminished after treatment ended [88]. Since many of the bacteria altered in autistic patients are important in the fermentation process that produces SCFAs, like butyrate, additional studies measured the SCFA content in autistic children. However, there are conflicting data as to whether or not this change in microbiota result in an increase or decrease in SCFAs in fecal samples [84,89]. Regardless, more studies would be necessary to determine if a change in SCFAs in the fecal matter is the result of poor absorption based on the increased gut permeability seen in autistic patients or excessive fermentation.

Interestingly, it has been hypothesized that elevated SCFAs in the circulatory system due to increased gut permeability or abnormal microbiota, may actually be detrimental to children with autism. Recent studies have shown that intracerebroventricular and peripheral injections of propionic acid (one carbon shorter than butyrate) during development results in autistic like behavior, including repetitive dystonic behaviors and object preference [90–94]. These behavioral changes were also observed to a lesser extent with butyric acid and acetic acid. However, this phenomenon has led to a rodent model of autism using propionic

acid to induce autistic symptoms [95,96]. In agreement with these studies is the increased risk of autism with prenatal exposure to valproate, an anti-seizure and mood-stabilizing drug that is a carboxylic acid HDAC inhibitor, like butyrate [97–100]. Given the large increase in autism diagnoses in recent years, there is a significant interest in understanding the etiology of the disease. While there are certainly many factors at play, propionic acid has come under some scrutiny. It has become an increasingly common food preservative due to its antimicrobial properties, which has increased our exposure to propionic acid [101]. In general, there is overwhelming evidence of the beneficial effects of butyrate and other SCFAs, but based on the autism literature some caution is warranted when considering prenatal exposure and the developing brain.

2. Conclusion

Butyrate is multi-functional molecule that has significant potential as a therapeutic for the brain, both in its pharmacologic and dietary form. Fig. 2 summarizes the various proposed mechanisms in which butyrate may influence brain health in a number of different neurological disorders. Pharmacologically, butyrate is capable of targeting many pathways with multiple mechanisms of action that are disease specific. The dietary sources of butyrate through a high fiber diet or a diet rich in natural sources of butyrate is a highly appealing approach, as it presents a simple and relatively low risk method to potentially improve outcomes in patients with brain disorders. Though much more research is needed to understand the effectiveness of these dietary interventions, they remain promising interventions that, if validated, may be used in the future in conjunction with traditional pharmacological treatments. As the current literature suggests, we can no longer overlook the importance of the gut-brain axis and nutrition in disease pathogenesis and treatment.

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