REVIEW ARTICLE

REEVALUATING THE HYPE: FOUR BACTERIAL METABOLITES UNDER SCRUTINY

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With microbiome research being a fiercely contested playground in science, new data are being published at tremendous pace. The review at hand serves to critically revise four microbial metabolites widely applied in research: butyric acid, flagellin, lipoteichoic acid, and propionic acid. All four metabolites are physiologically present in healthy humans. Nevertheless, all four are likewise involved in pathologies ranging from cancer to mental retardation. Their inflammatory potential is equally friend and foe. The authors systematically analyze positive and negative attributes of the aforementioned substances, indicating chances and dangers with the use of pre- and probiotic therapeutics. Furthermore, the widespread actions of microbial metabolites on distinct organs and diseases are reconciled. Moreover, the review serves as critical discourse on scientific methods commonly employed in microbiome research and comparability as well as reproducibility issues arising thereof.

Keywords: microbiota, microbial metabolites, flagellin, lipoteichoic acid, butyric acid, propionic acid

Introduction

“With every day, and from both sides of my intelligence, the moral and the intellectual, I thus drew steadily nearer to the truth, by whose partial discovery I have been doomed to such a dreadful shipwreck: that man is not truly one, but truly two.”

Robert Louis Stevenson, The strange case of Dr. Jekyll and Mr. Hyde

Throughout the history of mankind, tides have turned many a time on the concept of “health” [1]. While in ancient civilizations the importance of hygiene to avoid devastating diseases was already a well-known truth, this knowledge faded during medieval times yet to reemerge with full force owing to the life’s work of luminaries such as Ignaz Semmelweis [2], Louis Pasteur [3], and Alexander Fleming [4]. At the turn of the millennium, yet a new chapter has been opened allowing for undreamt-of possibilities by virtue of newly developed sequencing methods granting research in never before seen depth and width [5, 6]. The understanding of the importance of the microbial kingdom for human health has reached an unprecedented climax.

The microbial residents inhabiting a human, termed microbiota, in their entirety outnumber the eukaryotic cells by several decimal powers. The human gut alone is home to ten times more cells than the human body. The colon is by far the most heavily colonized organ in the body. Under physiological conditions, the host benefits from the intestinal microbiota as it fundamentally contributes to host health. The gut microbiota is processing nutrients, producing vitamins, acting as immunomodulator, and supersedes pathogens [7, 8].

The complex microbial community of the gut comprises a large and diverse population of bacteria, including symbionts, commensals, opportunists, as well as pathogenic parasites [5, 6]. Many of the compounds produced by these microbes enter circulation and thereby influence the function of distal organs and systems. Hence, many studies support the notion that the gut microbiota in itself is a distinct endocrine organ. Considering the vast amount

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and varieties of metabolites stemming from the microbiota, it is not surprising that alterations in this complex community can impact human health. What is more, a large body of evidence indicates that the intestinal microbiota not only has an impact on physical health, such as colitis or inflammation in general [9, 10], but also on behavior, cognition, and psychological health [11, 12]. In animal models, probiotic strains have been shown to alleviate depression-related behavior [13, 14]. Just recently, Dinan et al. [12] introduced the term psychobiotics for probiotics that have a beneficial impact on mental health. This further strengthens the concept of the microbiota-gut-brain axis, describing the bidirectional interaction of gut microbiota and the central nervous system [15]. The microbial metabolites present in the gut are vital for the microbiota as well, serving as surface protection, enabling movement or adhesion to surfaces, energy production, exchanging signals with the environment, and many more [16].

The body of research elucidating the characteristics of microbial substances is ever increasing. The use of these compounds ranges from the induction of disease models to the study of their therapeutic and even preventative value. Evidence has shown that countless microbial metabolites influence health and well-being of the host [17–19]. Hence, the number of microbial metabolites under study is vast and exceeds the capacities of this review. In the work at hand, four distinct metabolites, namely, flagellin, lipoteichoic acid (LTA), butyric acid (BA), and propionic acid (PA) are considered. They have gained interest in distinct areas of research, the most prominent ones will be described herein, and some have already been applied in clinical trials. This review is intended to give an impression on the wide array of bacterial metabolites used in research and highlight the ample potential they harbor for the understanding of disease and more importantly for the improvement of health.

Flagellin

Flagellin is the primary structural component of protofilaments that build the flagellum, the whiplike organelle that enables the bacterium to directed motility. The flagellin protein forms monomers of about 28–65 kDa that polymerize to protofilaments [20]. Gram-negative as well as gram-positive bacteria can express the genes encoding flagellin as part of the chemotactic regulon [21, 22]. Depending on the bacterium, the genes encoding the flagellin protein have been named differently. Flagellin has four domains, namely, D0, D1, D2, and D3. D0 and D1 are largely helical and mainly responsible for polymerization. For formation of polymers, the D0 domain of one monomer interacts with the D1 domain of the other monomer. Hence, D0 and D1 are required for motility and highly conserved even among widely diverse bacterial species. D1 also contains the amino acids that have been shown to be crucial for toll-like receptor 5 (TLR5) recognition and binding [21, 23–26]. D2 and D3 on the other hand contain rather variable regions that vary even between closely related bacteria. These domains are in the center of the flagellin protein and exposed at the outer flagellum surface [24].

Extracellular flagellin is recognized by TLR5, which is a pattern-recognition receptor and able to initiate innate immune responses in a MyD88-dependent pathway. This further leads to the activation of the transcription factor nuclear factor-κB (NF-κB). TLR5 is expressed on several immune cells, like dendritic cells, macrophages, and T cells, and on many types of epithelial cells [21, 27]. Noteworthy, activation of TLR5 by flagellin in epithelial cells not only leads to pro-inflammatory gene expression but also mediates antiapoptotic effects which help to maintain the integrity of the epithelial barrier [28]. The expression patterns of TLR5 are adapted to the normal localization of bacterial colonization. In the highly colonized colon, TLR5 is expressed on the basolateral surface of epithelial cells whereas in the lower airways, where bacterial colonization is low, TLR5 is expressed at the apical surface [29, 30]. TLR5 recognizes flagellin by residues in the conserved region of the protein. These residues are largely hidden in the protofilaments but easily accessible in the flagellin monomer. Hence, monomeric flagellin was shown to elicit much higher TLR5 agonism than flagelin polymers [24], and it is likely that monomeric flagellin operates as native TLR5 agonist because flagellated bacteria deliberately release it [31]. Interestingly, Helicobacter pylori [32] and Campylobacter jejuni [33] produce a mutated form of flagellin. The mutations are located in the conserved regions affecting only TLR5 activation but not polymerization and motility [34].

Intracellular flagellin, on the other hand, activates the intracellular receptors interleukin-converting enzyme protease inhibitor (IpaF) [35, 36] and Nod-like receptor apoptosis-inhibitory protein-5 (Naip5) [37–39]. This leads to the activation of caspase-1 via the inflammasome. For activation of IpaF and Naip5 by flagellin, a sequence of 35 highly conserved C-terminal amino acids is required [39]. The effects of flagellin on IpaF and Naip5 were mainly studied in macrophages. Collectively, the available evidence indicates that flagellin stimulates various immune cells via IpaF/Naip5 and TLR5, which leads to activation of the innate and adaptive immune system. Flagellin is thus highly immunogenic [40–42].

Salmonella strains are frequently used models in the research on flagellin. Studies using Salmonella strains that had lost the entire flagellum machinery showed that these strains are even more virulent compared to the flagellated strains in vivo [43]. Hence, the presence of flagellin seems to protect against bacterial dissemination by early immune-cell recruitment. Hawn et al. [44] observed that TLR5-lacking mice show a delayed recruitment of neutrophils after Legionella pneumophila infection, which results in a greater inflammatory pathology of the lung tissue at a later time point. In a murine model of Escherichia coli urinary tract infection, Andersen-Nissen et al. [45] reported a similar effect of TLR5 knockout. In this model, TLR5-knockout mice exhibited decreased inflammation...
in bladder tissue 2 days postinfection whereas, 5 days postinfection, they developed prominent inflammation in the bladder, and bacteria were detected in bladder and kidney. The beneficial effect of flagellin was also demonstrated by Vijay-Kumar et al. [46] in their study in which mice were orally infected with wildtype or agellated *Salmonella typhimurium*. The absence of flagellin led to a more severe clinical phenotype which was markedly reduced when animals were treated with intraperitoneal injection of flagellin 2 h before infection. The authors reported that agellated *Salmonella* greatly increased apoptosis in the mucosal epithelium, which may play a role in increased tissue injury and systemic invasion. In another study by the same authors, prophylactic administration of flagellin to mice was able to protect against oral dextran sodium sulfate (DSS)-induced colitis and against oral infection with rotavirus and γ irradiation [47].

Flagellin has attracted considerable interest in the field of immunology and vaccine development. Already in 1991, Newton et al. [48] constructed a flagellin carrying a protective epitope of the M protein of *Streptococcus pyogenes* type 5. *Salmonella* expressing this construct was used as live vaccine and were able to protect mice against *S. pyogenes* type 5 challenge. Cuadros et al. [49] used a flagellin-enhanced fluorescence protein fusion protein to show that flagellin is able to stimulate murine antigen presenting cells and induced cytokine secretion in vitro. In addition, immunization of mice with this fusion protein induced specific T-cell responses. The authors proposed flagellin fusion proteins to be potential constructs for the development of adjuvants and vaccines. Honko et al. [50] administered flagellin as adjuvant together with an antigen of *Yersinia pestis* intranasally to mice. Plasma immunoglobulin G (IgG) titers were greatly increased by flagellin treatment, and mice were protected against intranasal challenge with a virulent *Y. pestis* strain. The group also reported that the previous existence of anti-flagellin antibodies had no negative impact on flagellin’s adjuvant activity. This and other studies showed that the advantages of flagellin as vaccine are extensive: it is effective at low doses [51], it does not promote IgE responses [50], the antigen sequences can be inserted at various regions in the protein [52–54], and some fusion proteins were already shown to be safe and well-tolerated in human clinical trials [51, 55]. Even in aged mice, flagellin was shown to promote the adaptive immune response when used as adjuvant or vaccine although to a lower extent than in young animals [56, 57]. Asadi Karam et al. [58] demonstrated in mice that subcutaneous administration of flagellin–antigen fusion protein was more potent than flagellin admixed with the antigen to induce humoral and cellular immune responses against urinary tract infection.

Flagellin has also garnered considerable interest in the area of cancer research. Sfondrini et al. [59] showed that administration of flagellin to mice can influence tumor growth depending on the time of administration and tumor implantation, while early treatment with flagellin accelerated tumor growth, late treatment reduced tumor growth. Rhee et al. [60] used a mouse xenograft model of human colon cancer and reported that peritumoral administration of flagellin was able to increase tumor necrosis and thereby suppressed tumor growth. Lack of TLR5 or MyD88 expression on the other hand inhibited tumor necrosis and increased tumor growth. This was supported by the study of Cai et al. [61] in 2011 who reported that peritumoral administration of flagellin was able to inhibit tumor growth and increase tumor necrosis and neutrophil infiltration in a mouse xenograft model of human breast cancer. In contrast, Song et al. [62] observed that growth of gastric cancer cells was enhanced by flagellin treatment in vitro.

As already mentioned, TLR5 is polarized to the basolateral surface of the colon, so it should only be reached by flagellated bacteria which breach the epithelium or which are able to translocate their flagellin across the epithelium (e.g., *S. typhimurium* [63]). Hence, a disruption of the mucosal epithelial barrier, which can be seen in inflammatory bowel diseases (IBD), may lead to enhanced TLR5 activation [64]. Crohn’s disease is an IBD characterized by an aberrant and uncontrolled immune reaction against the normal gut microbiota [65]. Lodes et al. [66] reported that, unlike colitis patients, Crohn’s disease patients show a high antibody response to flagellin of commensal bacteria. Wallis et al. [67] also observed increased anti-flagellin antibodies in the serum of patients with ankylosing spondylitis, a pathology frequently associated with IBD. Rhee et al. [68] found that intracolonic administration of flagellin to either DSS-treated mice or mice with injured colonic mucosa initiated and aggravated inflammatory responses. The hypothesis of a decisive role of TLR5 in this pathology is corroborated by data from humans with dominant negative TLR5 polymorphism (TLR5-stop). Healthy TLR5-stop carriers were shown to have lower flagellin specific antibodies, and a negative association of TLR5-stop with Crohn’s disease, but not ulcerative colitis (UC), was observed [69]. Hence, reduced TLR5 function may offer some protection against Crohn’s disease development.

Flagellin has also gained growing interest in the area of allergy research due to its ability to promote Th2 but not IgE responses [50]. Schülke et al. [70, 71] reported that intraperitoneal administration of flagellin–ovalbumin fusion protein prevented intestinal allergy in mice. On the other hand, Wilson et al. [72] instilled flagellin together with ovalbumin into the airways of mice in which it induced strong allergic airway responses to ovalbumin. They also found high titers of anti-flagellin antibody in sera of asthmatics.

For research purposes, flagellin has been administered by various routes (e.g., oral, intranasal, and intraperitoneal) and in various forms such as purified flagellin, fusion-proteins, or whole flagellin-expressing bacteria. Most research on flagellin has been conducted in mice, but in recent years, flagellin has also been tested in clinical trials [51, 55, 73, 74]. Furthermore, new combinations and fusion proteins with flagellin are being tested in animals, especially in poultry vaccination [75]. The search for new vaccines and tolerable adjuvants is ongoing, and flagellin
seems to have great potential for the use in this area. Also the role of flagellin in cancer and allergies is still unclear, and further research is needed to elucidate the elusive properties of this protein.

**Lipoteichoic acid**

LTA is a major cell wall constituent of gram-positive bacteria, a so-called surface-associated adhesion amphiphile [76]. The basic structure is composed of a soluble polymer attached to the cell membrane via a diacylglycerol. The polymer consists of polyhydroxy alkane units, such as ribitol and glycerol. The sequence of glycerol and ribitol varies between species [77]. LTA is mainly released after bacteriolysis, an important matter of concern with the use of β-lactam antibiotics. It specifically binds to cluster differentiation 14 (CD14) or Toll-like receptor 2 (TLR2) and, thus, triggers the release of pro-inflammatory mediators and cytokines such as tumor necrosis factor α, interferon γ, and interleukins 1, 5, 6, and 8 [76, 78]. However, it can also nonspecifically bind to membrane phospholipids. Albeit having less pro-inflammatory potential than lipopolysaccharide, LTA has been reported to induce arthritis, nephritis, toxic shock syndrome, uveitis, encephalomyelitis, meningeval inflammation, and periodontal lesions in animal studies. Moreover, it can trigger cascade reactions resulting in septic shock and multi-organ failure. Strikingly, 50% of cases of sepsis and septic shock are caused by gram-positive bacteria [76], with *Staphylococcus aureus* being one of the most prevailing organisms causing nosocomial infections. More importantly, the emergence of multidrug resistant clones of methicillin-resistant *S. aureus* (MRSA) is an ongoing issue far from being under control [79]. LTA is furthermore prone to act synergistically with other microbial compounds such as endotoxin and peptidoglycan, thereby either enhancing or diminishing the inflammatory response [80].

In the past, issues have been raised concerning the purity of LTA preparations used in research [81]. Commercial preparations were found to contain trace amounts of endotoxin, yielding them inappropriate for experiments [82]. While commercial suppliers indicate purity of their LTA products, researchers are advised to verify their results via LPS blocking agents [83]. In 2001, Morath et al. [82] called attention to the issue of inactivation of LTA over the course of the purification process. The group demonstrated an improved purification process, yielding pure (>99%) biologically active LTA. Seo et al. [84] furthermore demonstrated how improved purification methods preserve LTA activity. Using this preparation, the group was able to induce a distinct immune response via LTA, an effect not evoked by LTA preparations inactivated via three different methods.

Recent reports suggest an ability of *S. aureus* to adhere to and invade the blood brain barrier (BBB), leading to meningeal inflammation and brain abscess formation including significant levels of brain bacterial counts [85]. Considering the high prevalence of infections caused by gram-positives and the capacity of such species to trespass the BBB, an analysis of possible consequences on host brain and mental health is desirable [79, 85]. Calling to mind the widespread use of gram-positive bacteria in probiotic formulations, the reevaluation of LTA regarding brain health and behavior should gain even more precedence.

**Butyric acid**

BA is a short chain fatty acid (SCFA) with four carbon atoms. BA and other SCFAs are produced by the resident gastrointestinal microbiota via fermentation of dietary fibers [86]. Resistant starch [87], inulin [88], and oligofructose [89] have been shown to increase the fecal content of BA-producing bacteria. Therefore, they have been suggested to be very efficient butyrogenic substrates. Besides colonic production, BA is also frequently used as a food additive [90]. As BA is the primary energy source for colonocytes, the majority is metabolized in the gut and only small amounts are processed in the liver. Thus, concentrations in the general circulation are comparatively low [91]. The majority of BA-producing bacteria are anaerobic gram-positives. Fecal sample analyses indicate members of clostridiala clusters IV and XIVa as the most important and abundant anaerobic BA-producing bacteria [92]. Transport of BA is cell type-specific; nonionic diffusion and monocarboxylated transporters (MCT) are two of the so far identified transport mechanisms of enterocytes [86]. The primary receptors for SCFAs are the G protein-coupled receptors (GPR) 41 and 43, though BA exhibits stronger affinity to GPR41 [93]. GPRs are cell surface receptors that sense extracellular molecules and activate signal transduction pathways in the cell upon binding. Both GPR41 and GPR43 are not only expressed in enterocytes but also in adipocytes. GPR41 is furthermore expressed in the sympathetic nervous system [94] whereas GPR43 is highly abundant in immune cells [93].

While BA has been in the spotlight of various areas of research by virtue of its touted beneficial effects, one should be aware of the controversies of data in this regard. Under healthy conditions BA stimulates growth and differentiation of epithelial cells and colonocytes. In addition, BA decreases apoptosis of normal colonocytes [95]. Contrary to healthy conditions, BA induces apoptosis in human colon carcinoma cells. Due to these controversial actions, the term “butyrate paradox” was coined [96]. Evidence suggests that the concentration-dependent effect of BA on carcinoma cells is mainly exerted by the histone deacetylase inhibiting properties of BA. Histone deacetylases are important epigenetic regulators of gene transcription and the target for many chemotherapeutic drugs [97]. BA sparked the interest of cancer research already in the 1980s when sodium butyrate was used for *in vitro* studies in human cancer cells [98]. In various cancer cell lines, obtained from colon [99], breast [100], bladder [101], and
prostate [102] cancer, sodium butyrate and other derivatives of BA exerted anticancerogenic effects. The studies indicate that the effects of BA on cancer cell lines are due to induction of apoptosis, tumor cell differentiation or simple prevention of cell proliferation. Weir et al. [103] reported recently that BA-producing bacteria are reduced in the stool of colorectal cancer patients. Noteworthy, not all studies observed a beneficial role of BA in colorectal cancer. Therefore, it was suggested that the time point of administration or the state of the cancerous cells plays an important role in the anticancerogenic actions of BA [104]. Nevertheless, BA has reached prominent interest in cancer research, and studies on this topic are ongoing.

Oxidative stress is a further factor contributing to carcinogenesis and inflammation. Hamer et al. [105] showed that daily sodium butyrate enemas in healthy patients increased the amount of the antioxidant glutathione, which points towards an antioxidative effect of BA.

BA has also been proposed to improve intestinal barrier function by upregulating mucin expression [106] and proliferation of epithelial cells. An impaired integrity of the gut barrier plays an important role in numerous infectious and gut diseases [107]. In UC patients, butyrate concentrations are lower than in healthy controls [108]. It has even been implied that butyrate metabolism is impaired in these patients [109]. Butyrate enemas have been reported to exert beneficial effects in UC and experimental colitis [110, 111]. Recently, Vieira et al. [112] showed that oral administration of sodium butyrate in a mouse colitis model is able to ameliorate mucosal damage and decrease inflammation. Hence, oral butyrate supplementation could be beneficial in UC patients. An additional anti-inflammatory property of BA is the inhibition of transcription factor NF-κB activation. Due to this action BA reduces the expression of pro-inflammatory genes [113]. It has been shown that NF-κB is frequently activated in epithelial cells of the inflamed intestinal mucosa and in macrophages, suggesting a role in IBD, such as Crohn’s disease and UC [114]. In line with this contention, luminal administration of BA has been proposed to ameliorate symptoms in UC patients and reduce inflammation [115].

Besides these local effects, BA has in addition been shown to exert beneficial systemic actions. In the 1990s, clinical trials with orally administered sodium phenylbutyrate were performed in sickle cell disease and β-thalassemia patients. The reports showed that sodium phenylbutyrate was able to increase fetal hemoglobin production [116, 117]. Although novel butyrate derivatives are currently investigated in clinical trials, an optimal treatment regimen has not yet been identified [118, 119]. Furthermore, sodium phenylbutyrate has been tested in patients with urea cycle disorders where it acted as an ammonia scavenger [120].

The field of neurology has shown growing interest in the use of BA as well. In 2007, Schroeder et al. [121] demonstrated that daily intraperitoneal injections of 1.2 g/kg sodium butyrate to mice exert antidepressant-like effects in the tail suspension test. The same dose of BA was able to improve neurological parameters in a mouse model of Huntington’s disease [122]. In rats subjected to cerebral ischemia, Kim et al. [123] observed that subcutaneously injected sodium butyrate enhanced incorporation of bromo-2’-deoxyuridine in the brain. Hence, sodium butyrate was suggested to stimulate neurogenesis after ischemic injury. Interestingly, it has been reported that fecal SCFA levels including BA are elevated in children with autism spectrum disorder [124]. Takuma et al. [125] showed that autism-like behavioral abnormalities induced prenatally in mice could be ameliorated by chronic intraperitoneal treatment with sodium butyrate. It should not go unnoticed, though, that BA has also been reported to enhance colonic pain sensitivity in rats [126].

The data regarding obesity and BA are still controversial [127]. In 2009, Gao et al. [128] reported that, in a mouse model of metabolic syndrome, oral supplementation of a high-fat diet with butyrate was able to attenuate the development of obesity and insulin resistance. Further studies demonstrated that, even in humans, diets that elevated fecal butyrate concentrations proved beneficial [129, 130]. Recently, Fernandes et al. [131] reported that fecal SCFA concentrations, including BA and PA, were significantly higher in overweight or obese patients than in lean subjects. This supports the hypothesis that colonic fermentation is altered in overweight or obese adults. However, the role of BA in obesity is still not fully elucidated [131] although an increasing number of studies points towards a beneficial effect of BA in obesity-related factors [132–135]. Interestingly enough, BA-producing bacteria were found to be decreased in faecal samples of type-2 diabetes patients [136].

For research purposes, especially in the field of neurology, BA is often administered via a parenteral route to exert stronger effects at smaller doses [121–123, 125]. However, in experimental models of colitis, the oral [112] and rectal [111, 113] route of application proved to be most successful. Therefore, supplementation of the diet with BA as well as prebiotic supplementation to promote BA production could be of interest to other research fields as well. Currently, in the majority of therapeutic attempts, BA is administered orally [137] or as an enema [115, 138]. Although the handling of BA is easy and there is an abundance of BA salts available, the taste and odor of BA if given in acidic form are unpleasant, complicating its use particularly in children. Nevertheless, the body of evidence concerning the beneficial effects of BA is continuously growing and, according to the data available, BA has great potential for future therapeutic applications.

Propionic acid

BA’s “little brother” is the SCFA PA, bearing only three carbon atoms. While a lot of research focused on the role of BA, little has been done to let PA emerge from the shadow of his elder and reveal its potential. PA is a common constituent of the modern human diet owing to its ac-
tions as a food preservative due to its antifungal [139] and antimicrobial [140] effects. However, the majority of PA present in humans, like BA, is produced by the colonic microbiota through fermentation of dietary fibers [141]. Currently, not all PA-producing bacteria have been identified, though the ability of PA production seems not to be limited to one phylum. PA producers are for instance Bacteroides fragilis [142] and Propionibacterium [143]. The transport of PA by enterocytes is similar to that of BA and involves, for instance, MCT transporters [86]. Amidst SCFAs, PA is the prime ligand for GPR41 and GPR43 [93]. As already stated, GPR41 is expressed by enterocytes, adipocytes, and the sympathetic nervous system [94]. GPR43 is expressed in immune cells, adipocytes, and the intestine [93]. These expression patterns concord with the main field of action of PA. While the majority of BA is metabolized in the intestine, PA proves less favorable as an energy source for colonocytes. Consequently, much higher concentrations of PA are being taken up and metabolized by the liver [91]. Contrary to humans, PA is used as a major gluconeogenic substrate in ruminants [144]. Such a diversity of action modes scattered across species complicates the interpretation of findings, although it also sheds light on contradictory results obtained in various PA studies. As a substantial body of research conducted on PA converges on its effects on satiety, energy intake, and other obesity-related factors, the information presented herein focuses on studies in non-ruminants.

Animal experiments indicate that PA is playing a role in decreasing cholesterol and fatty acid levels in the liver [145]. Lin et al. [146] compared the PA concentrations required to lower cholesterol in rat and human hepatocytes. The susceptibility of human hepatocytes to PA was markedly smaller than that of rat hepatocytes, and the PA concentrations needed to inhibit cholesterol synthesis were around 10–20 mmol/l. However, it requires consideration that the physiological concentration of PA in the portal vein of about 0.1 mmol/l is substantially lower than the concentration required to attenuate cholesterol levels [141]. In 2004, Xiong et al. [147] reported increased plasma leptin levels in mice following acute oral administration of sodium propionate. Enhanced expression of this anorexigenic hormone in human adipose tissue was also reported by Al-Lahham et al. [148] in 2010. Both studies suggest an involvement of GPR41 in this process. Furthermore, Ge et al. [149] demonstrated reduced lipolysis in murine adipocytes treated with PA. In addition, GPRs are expressed by enteroendocrine cells which are capable of transducing information via gut hormones such as PYY or GLP-1. In line with this hypothesis, Psichas et al. [150] recently demonstrated increased PYY and GLP-1 concentrations in portal vein plasma of mice and rats subsequently to intracolonic administration of PA. In GPR43-deficient mice, PA treatment was unable to enhance the release of PYY and GLP-1.

To date, it remains elusive whether PA exerts beneficial effects on IBD. As far as we can tell, no study has been performed using enemas with PA as the sole active ingredient, PA being tested only in SCFA mixtures. The treatment of UC with SCFA enemas appeared promising in some studies [159, 160], albeit others saw only small trends towards an improvement [115, 161]. Since the available studies used varying concentrations, volumes, and treatment regimes, the data at hand are inconclusive, let alone a number of intersubject differences. Anyhow, Tana et al. [162] observed high fecal PA and acetic acid concentrations in irritable bowel syndrome patients; these parameters correlate with the severity of symptoms and negative emotions.

PA has also been reported to have beneficial effects on colon cancer cell growth [163] and to induce apoptosis in colorectal cancer cells, albeit to a lesser degree than BA [164]. In 2002, Jan et al. [165] and Hinnebusch et al. [166] observed in independent studies antiproliferative effects of PA in colon cancer cells. Jan et al. [165] found that supernatants of three strains of the Propionibacterium genus caused colorectal cancer cells to die. The cytotoxic effect was attributed mainly to PA and acetic acid which elicited apoptosis in the colorectal cancer cell line. Hinnebusch et al. [166] treated colon carcinoma cells with SCFAs including BA and PA. While BA and PA both induced significant histone hyperacetylation, the effect was more pronounced with BA. The same pattern was observed regarding the inhibition of growth rate of cancer cells, that

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is, BA was more effective than PA. However, induction of cell differentiation was more pronounced with PA treatment. Yet in contrast to Jan et al. [165], Hinnebusch and colleagues [166] observed an induction of apoptosis only in cells treated with BA.

In the context of PA, attention has to be drawn to propionic acidemia, which is a metabolic disease characterized, among others, by very high PA concentrations in the body. The disease is caused by malfunction of propionyl-CoA carboxylase and involves symptoms such as vomiting and mental retardation [167, 168]. Those symptoms can also be seen in other organic acidemias [169]. Though liver transplantation is able to alleviate many of these symptoms, it has little effect on PA concentrations [170].

Adverse effects of PA have also been observed with respect to behavior and mental health. In 2007, MacFabe et al. [171] demonstrated induction of some symptoms of autism spectrum disorder following intraventricular infusion of PA in rats. Furthermore, they found increased oxidative stress markers in the brain and reported the presence of neuroinflammation indicators. Similar experimental setups support these findings and provide more evidence for abnormalities in brain and behavior [172–174]. These data fit well to the observation that the fecal SCFA concentrations are elevated in children with autism spectrum disorder [124]. In an experimental model of chemically induced propionic academia, young rats were treated subcutaneously with buffered propionate for three weeks. This treatment caused a delay in some attributes of physical development. In addition, a higher number of rearings in a repeated open field test and an aggravated avoidance response in the shuttle-avoidance task were observed 3 weeks after discontinuation of treatment [175]. Hence, the authors hypothesize that chronic PA treatment causes long-term behavioral deficits in aversive memory and habituation to novelty.

Remarkably enough, PA has also been demonstrated to have pro-inflammatory effects. In gingival inflammation, periodontal bacteria release high concentrations of SCFAs and induce inflammation of gum tissue. There is evidence that PA is the major SCFA produced in this process in humans [176]. Direct application of a SCFA mixture including PA, acetic, lactic, and formic acid to healthy patients elevated subgingival temperature and neutrophil emigration. The same effect was observed following supplementation of food with high amounts of the aforementioned mixture [177].

Most of the PA effects proposed are based on animal experiments only. Moreover, many studies performed in humans used a SCFA mix [161] or prebiotics such as resistant starch [156]. In consequence, it is not possible to separate the effects of the SCFAs and to analyze any synergistic or antagonistic actions. Considering the current state of knowledge, further studies in humans and with human-derived cells are necessary to elucidate the effects of PA in health and disease.

Conclusion

The amount of data generated by contemporary microbiome research is vast and ever evolving. To date, the microbiome’s pivotal impact on host health is unequivocal. Even more so, the complexity of the microbiota–host interactions exceeded the expectations of most. Certainly, the founding fathers of today’s pro- and prebiotic therapeutic approaches would be proud. Alfred Nissle, who developed the E. coli Nissle 1917 probiotic Mutaflor® in 1917 [178], or the Austrian researcher Friedrich Petuely, who in 1960 filed a patent for the discovery of lactulose as “bifido factor,” a prebiotic specifically enhancing growth of Bifidobacteria strains [179], would be very content.

Vital questions, however, remain. Even though modern sequencing methods allow for the generation of massive amounts of data, the definition of a healthy or physiological microbiome is still pending. While the enterotype hypothesis seemed promising, evidence suggests that the picture is not as clear-cut as initially hoped for. Recent research indicates that function rather than phylogenetic aspects is the key factor for a healthy microbiota. Furthermore, the interleaved crosstalk between host and microbiota via microbial metabolites is still little understood. The review at hand serves to demonstrate how a single metabolite can act as the good, the bad, and the ugly, all depending on the circumstances and location of its action.

As long as the scientific community stays attentive, is not blinded by the light of possibilities, and stays focused on the rationality of the approaches pursued, research in this area offers great opportunities to further the understanding of health and disease. Moreover, it proposes never before seen chances for the prevention and treatment of diseases within and beyond the gastrointestinal tract.

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