Role of gut microbiota in the 5-hydroxytryptamine signal transduction mechanism

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ABSTRACT

As a neurotransmitter, 5-hydroxytryptamine (5-HT) plays a variety of regulatory roles in the brain, including affecting mood, memory, circadian rhythm, and other aspects. However, the brain contains only approximately 10% of the 5-HT in humans. Ninety percent of the 5-HT is produced and stored in the gut. 5-HT in the gut is involved in the regulation of irritable bowel syndrome (IBS), colitis, obesity, and other diseases. Therefore, a comprehensive understanding of the bioactivity of 5-HT is necessary. The gut microbiota has been reported to affect 5-HT synthesis and function, but the exact mechanism remains unclear. This review focuses on the production of 5-HT, its relationship with gut microbiota, its mechanism of action in different disease progression processes, and the biological effects of its derivative 5-hydroxyindoleacetic acid.

Key words: gut microbiota, 5-hydroxytryptamine, inflammation, 5-hydroxyindoleacetic acid

INTRODUCTION: GUT MICROBIOTA

As a research hotspot in recent years, the gut microbiota is active in nutrition, immunology, neurology, and other fields. Due to the wide variety and large number of gut microbiotas, people are driven to study their interaction with humans. Generally, the bacteria in the human intestine are mainly from *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, *Verrucomicrobia*, and *Fusobacteria*, among which *Firmicutes* and *Bacteroidetes* dominate the ecological niche.[1] For a long time, it has been believed that the fetus is sterile when it is still in the mother, but researchers have found bacteria and other microorganisms in the placenta, umbilical cord, amniotic fluid, and even the meconium of newborns.[2,3] It can be said that the gut microbiota accompanies life, and it will constantly change dynamically with changes in individual diet and living habits regardless of infancy, youth, or old age.[4] It is difficult to elaborate on the mechanisms by which the gut microbiota affects humans because the gut microbiota may play a regulatory role either directly or indirectly. Kuziel *et al.*[5] divided the direct regulation of microorganisms into three situations: small molecule metabolites, ligands for pattern recognition, and molecules that act by influencing adaptive immunity. Because of the existence of various complex regulatory mechanisms, the gut microbiota is also known as an important source of metabolites in humans. Currently, more in-depth studies include short-chain fatty acids (SCFAs) such as acetic acid, propionic acid and butyric acid, 5-hydroxytryptamine (5-HT, serotonin) metabolized from tryptophan, and a variety of bile acids.

Under normal circumstances, SCFAs can be metabolized by intestinal bacteria through dietary fiber and interact with the G protein-coupled receptor (GPCR) 41, GPCR43, and GPCR109A on the surface of intestinal epithelial cells and immune cells, which play an important role in maintaining the homeostasis of the internal environment.[6] In addition, branched-chain fatty acids (BCFAs), such as isobutyrate, 2-methylbutyrate, and isovalerate, as well as propionate intermediates lactic acid and succinate, can still be produced by some bacteria and exert biological effects on the human despite their relatively low abundance.[7]

Tryptophan in humans is mainly involved in metabolic pathways, including kynurenine, indole derivatives, and 5-HT, which have been proven to be directly or indirectly controlled by gut microbiota. The critical role of the gut microbiota in stimulating the activity of indoleamine 2,3-dioxygenase 1 (IDO1), a rate-limiting enzyme of kynurenine metabolism, has been clearly demonstrated, and the end products of its metabolism can be associated with host neurotransmission, inflammation, and immune responses.[8] Indole derivatives are molecules that are directly converted by the gut microbiota. For example, indole-3-aldehyde (IAld), indole-3-acid-acetic acid (IAA), indole-3-propionic acid (IPA), indole-3-acetaldehyde (IAAld), and indole-acrylic acid are all ligands of aryl hydrocarbon receptor (AhR).[9,10] This signal often acts on epithelial cell renewal, barrier integrity, and many immune cells.[11] 5-HT is also an important gastrointestinal signaling molecule that can transmit signals from the intestine to the nervous system by acting on at least fourteen 5-HT receptor subtypes,[12] affecting intestinal peristalsis and movement, secretion,[8] platelet aggregation,[13] cardiac function[14] and immune response.[15]

5-HT PRODUCTION PATHWAY

Most of the 5-HT in the human body is synthesized, stored, and released by a special intestinal epithelial cell subtype, enterochromaffin cells, which usually exist in the intestinal mucosal epithelium.[12] Enterochromaffin cells use Trp hydroxylase 1 (TpH1) to generate 5-hydroxytryptophan (5-HTP), which then generates 5-HT under the action of aromatic amino acid decarboxylase.[8] In addition, a small part of 5-HT is produced in the brain as a neurotransmitter through Trp hydroxylase 2 (TpH2) in serotonergic neurons, which plays an important role in neuroregulation. 5-HT in the gut also mediates cell-to-cell signaling, although 5-HT produced in the periphery cannot enter the brain because of the blood-brain barrier. Enterochromaffin cells act as sensors in the gut. When chemical changes or mechanical stimuli occur in the environment,[16] entero-chromaffin will capture the signal and selectively release 5-HT. Studies have shown that the basement membrane is the main site of 5-HT release in enterochromaffin cells, as in most types of enteroendocrine cells.[17] The secreted 5-HT reaches the lumen of the gastrointestinal tract and the bloodstream, where it is absorbed and concentrated by platelets. Because platelets lack the TpH to produce 5-HT, they cannot synthesize 5-HT, so 5-HT entering the circulatory system is the only source of 5-HT.[12] 5-HT that arrives in the gut will exert its biological function through 5-hydroxytryptamine receptors (5-HTR). For example, motor neurons express 5-HTR1A, 5-HTR1B/1D, 5-HTR2A, 5-HTR2B, 5-HTR3 and 5-HTR4, and intestinal muscle cells express 5-HTR1B/1D, 5-HTR2A, 5-HTR2B, 5-HTR4 and 5-HTR7. Primary afferent neurons (PANs) that affect intestinal sensitivity express 5-HTR3, 5-HTR4, and 5-HTR7.[18] Because of the existence of multiple receptors, 5-HT can play different regulatory functions. In addition, since there is no extracellular enzyme for 5-HT degradation, transporters are needed to recycle 5-HT and then allow intracellular enzymes to play a role in degradation. The serotonin reuptake transporter (SERT) serves this function and is expressed in almost all intestinal epithelial cells to transport 5-HT through a sodium- and chlorine-dependent mechanism, thereby removing excess 5-HT from the interstitium.[19–22] This clearance is important for 5-HT-mediated signaling.

5-HYDROXYINDOLEACETIC ACID (5-HIAA)

5-HIAA is a product of 5-HT metabolism by monoamine oxidase (MAO). Early studies focused on the correlation between the decreased content of 5-HIAA in cerebrospinal fluid (CSF) and mental diseases such as suicide and depression. The most common biomarker of suicide was the decreased concentration of 5-HIAA in CSF of suicide cases.[23] It was also found that the level of 5-HIAA in CSF was lower in suicide attempters, especially in violent suicide attempters.[24] The accumulation of HIAA in manic depressive patients may decrease in both manic and depressive periods.[25] In addition, lower CSF HIAA levels, a previous history of depression, and greater functional disability are associated with a higher risk of depression in Parkinson's disease (PD).[26] Here, 5-HIAA reflects the turnover and metabolism of 5-HT, especially the decreased content in CSF, which indicates the deficiency of the central nervous system (CNS) 5-HT function, which is the basis of some recurrent psychopathological disorders.[27] 5-HIAA is a detection indicator of 5-HT, which also appears in the study of gastrointestinal and pancreatic carcinoid endocrine tumors.[28] Previous studies have found elevated plasma 5-HIAA concentrations in patients with metabolic syndrome, suggesting that 5-HT may play an important role in the development of cardiovascular disease in patients with metabolic syndrome. Fasting blood glucose was positively

correlated with the logarithm of plasma 5-HIAA concentration, while high-density lipoprotein cholesterol was negatively correlated.[29] Similarly, the authors suggested that 5-HIAA is a level indicator of 5-HT, suggesting that 5-HT mediates vasoconstriction and induces platelet activation, which may promote atherosclerosis.

5-HIAA is considered an indicator of 5-HT degradation, but its function as a metabolite itself has not been experimentally proven. It has been considered a waste metabolite of 5-HT and has no biological activity. Recent experimental studies have revealed novel biological functions of 5-HIAA. Rosser *et al.*[30] found that butyrate supplementation inhibited arthritis by increasing the level of the 5-HT-derived metabolite 5-HIAA and activating the AhR, a newly identified transcriptional marker of regulatory B-cell function, as well as inhibiting germinal center B-cell and plasmablastic differentiation. Giovanni *et al.*[31] found that 5-HIAA is a GPCR35 ligand and neutrophil chemoattractant and determined the role of 5-HIAA produced by platelets and mast cells in cell recruitment to inflammatory sites and bacterial clearance. Interestingly, in a transgenic APPSWE mouse Alzheimer's disease (AD) model and a phosphamide-induced brain neutral endopeptidase inhibition mouse model, repeated chronic treatment with 5-HIAA significantly reduced brain amyloid β-protein (Aβ) and improved the cognitive ability of APPSWE mice. 5-HIAA inhibited the mitogen-activated extracellular signal-regulated kinase/extracellular signal-regulated kinase (MEK/ERK) pathway. This mechanism appears to interact with the mitogen-activated protein kinase (MAPK) pathway.[32] Since then, 5-HIAA should no longer be regarded as an inactive metabolite of 5-HT, and the focus should be shifted to its biological function. The next step is to study its extensive and sufficient mechanism.

GUT MICROBIOTA AFFECT 5-HT PRODUCTION

It has been shown that 5-HT synthesis in the gut is regulated by the gut microbiota (Figure 1). Using TpH1 knockout mice, it was found that the level of 5-HT in the gut and blood decreased by more than 90%,[33] indicating that less than 10% of peripheral 5-HT is directly through the synthesis of microorganisms in mice or TpH2-mediated biosynthesis. By using germ-free (GF) mice, it can be found that the production process of 5-HT in the colon of GF mice is impaired and the concentration of 5-HT in the blood is decreased,[34] which verifies that the gut microbiota is involved in 5-HT synthesis to a certain extent. Current studies suggest that gut microbiota may induce the transcription of TpH1, thereby affecting the production of 5-HT in the gut. In addition, SCFAs produced by microbiota have

also been reported to induce the expression of TpH1 in endocrine tumor BON and RIN14B cells.[7] Some secondary bile acids transformed by microorganisms, such as deoxycholic acid, also stimulated the production of 5-HT.[34] *In vitro* culture, *Corynebacterium spp.*, *Streptococcus spp., Escherichia coli, etc.,* can synthesize 5-HT.[35] However, this process does not depend on TpH, instead, tryptophan decarboxylates to form tryptamine. At present, there is no gut microbiota that can directly synthesize 5-HT in the human intestine. However, Valles-Colomer *et al.*[36] found that nearly 20% of gut bacteria have the potential to synthesize 5-HT using metagenomic data from a large cohort, which also provides direction and guidance for further exploration of the role of gut microbiota in the 5-HT pathway in the future. Selective serotonin reuptake inhibitors (SSRIs), a class of drugs commonly used to treat patients with depression, tend to increase the concentration of 5-HT in the environment by blocking reuptake during cellular signaling. Lukić *et al.*[37] found that the use of various selective serum reabsorption inhibitors in mice significantly changed the microbial composition in the body, and supplementation of *Ruminococcus flavefaciens* with duloxetine weakened the antidepressant treatment effect of the drug. This effect may be induced by changes in synaptic and mitochondrial gene expression to alter neuronal function.

5-HT AND DISEASE

Irritable bowel syndrome (IBS)

As a common gastrointestinal disease, IBS is a cluster of symptoms caused by a variety of diseases. Its clinical manifestations are usually intermittent episodes of abdominal pain, abdominal distension, and changes in bowel habits and stool traits. Since patients with this disease often experience pain or discomfort, their quality of life is significantly reduced.[38–40] At present, the pathogenesis of the disease is not clear, so it is generally only symptomatic treatment, that is, treating the symptoms rather than the root cause. Some pathophysiological studies on IBS have found that patients' intestinal motility capacity changes and sensitivity increases, and they are more likely to be "stressed" by some external factors that cause gastrointestinal discomfort. Among these factors are changes in mood, such as depression, anxiety, and depression, which have been shown to be related to the 5-HT system.[41] Coates *et al.*[22] found that patients with ulcerative colitis (UC), irritable bowel syndrome with diarrhea (IBS-D), and irritable bowel syndrome with constipation (IBS-C) had decreased signals regarding 5-HT in rectal samples compared with healthy controls (HCs). Including mucosal 5-HT, *TpH1* mRNA, 5-HT transporter mRNA, and 5-HT transporter immunoreactivity were significantly reduced. The researchers believe that these results suggest that alterations in the 5-
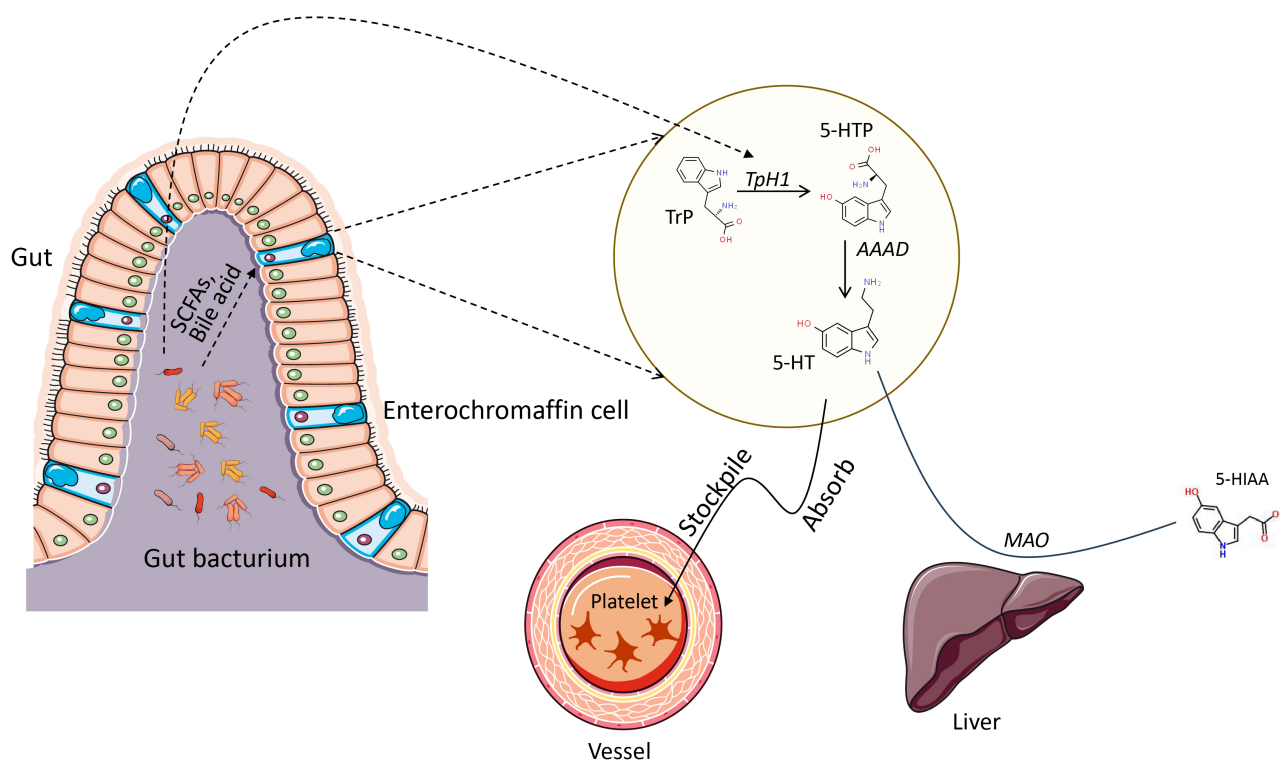
Figure 1. Gut microbiota stimulates 5-HT production by producing short-chain fatty acids and bile acids. In intestinal enterochromaffin cells, tryptophan generates 5-HTP under the action of the TpH1 enzyme and is then metabolized to 5-HT by AAAD. In addition to being stored in intestinal enterochromaffin cells, 5-HT also enters the intestinal lumen and blood and is absorbed by blood platelets. Furthermore, 5-HT is also metabolized to 5-HIAA by MAO in the liver. Although 5-HIAA has long been considered a metabolic waste, its biological functional activity has not been realized until recently. SCFAs: short-chain fatty acids; TpH1: Trp hydroxylase 1; 5-HTP: 5-hydroxytryptophan; AAAD: Aromatic L-amino acid decarboxylase; 5-HT: 5-hydroxytryptamine; MAO: monoamine oxidase; 5-HIAA: 5-hydroxyindoleacetic acid.

HT system in the intestinal wall may directly contribute to the symptoms associated with IBS and other gastrointestinal dysfunction. However, studies have reported an increasing trend of 5-HT levels in the colon of patients with IBS, possibly due to the decreased expression of SERT in the colon,[42] which makes monoamine oxidase A (MAOA), which should be responsible for degrading 5-HT in the colon tissue, unable to play its role. SERT knockout mice also suffer from watery diarrhea, enhanced colonic motility, and visceral allergy.[21] These results suggest that when the absorption capacity of 5-HT by colonic cells decreases, the concentration of 5-HT increases, which may promote the development of IBS.

Cao *et al.*[43] used the supernatant of *Lactobacillus rhamnosus GG* to treat a rat model of post-infectious irritable bowel syndrome (PI-IBS) induced by *Campylobacter jejuni* infection and found that SERT mRNA and protein levels in intestinal tissues were higher than those in control and phosphate buffered saline (PBS) gavage rats. Gao *et al.*[44] found that gavage of fecal supernatant from IBS-D patients to young mice resulted in a significant decrease in SERT expression and an increase in 5-HT levels in the colonic mucosa of mice. The mechanism is that endotoxin in the IBS

environment stimulates the synthesis and release of prostaglandin E2 (PGE2) from enteroblasts, which then acts on intestinal epithelial cells to downregulate SERT, leading to an increase in mucosal 5-HT. To test this hypothesis, mice were intragastrically injected with the lipopolysaccharide (LPS)-containing antagonist lipopolysaccharide-Rhodobacter sphaeroides (LPS-RS) or with LPS-removed fecal supernatant and were found to prevent the downregulation of SERT induced by IBS-D fecal supernatant. These studies suggest that intestinal microbiota disorder can affect the normal function of 5-HT signaling, which provides a new idea for the treatment of IBS.

Obesity

With the continuous improvement of living standards and changes in eating habits, obesity has become an urgent problem to be solved worldwide.[45] Obese people tend to have a higher risk of metabolic diseases, including heart disease, atherosclerosis, diabetes, and fatty liver, than HCs. Since obesity is caused in most cases by an imbalance between energy intake and expenditure, it is important to understand the causes of excess nutrition and the factors driving food intake.[46] It is known that 5-HT produced in the brain uses CNS to reduce appetite to regulate energy balance. Since 5-HT

produced in the periphery cannot cross the blood-brain barrier, although the gut microbiota can affect the production of 5-HT in the gut, it is still not directly involved in the regulation of the CNS. However, there is considerable evidence that peripheral 5-HT is closely related to energy metabolism. Simansky *et al.*[47] used rats as a model and found that rats achieved satiety faster in a behaviorally specific manner by injecting 5-HT in the periphery. Crane *et al.*[48] used mice with *TpH1* gene knockout or mice with LP533401, a small molecule that inhibits gut-derived TpH1, to feed a high-fat diet (HFD) and found that obesity, insulin resistance, and non-alcoholic fatty liver disease (NAFLD) could be prevented, while brown adipose tissue (BAT) exhibited greater energy expenditure. The specific mechanism is to protect or reverse the development of HFD-induced obesity and blood glucose by activating uncoupling protein-1 (UCP1)-mediated thermogenesis. Related experiments in *Caenorhabditis elegans* have shown that, unlike the regulation of feeding, the regulation of fat by 5-HT relies on a 5-HT-gated chloride channel encoded by mod-1 and a GPCRs initiated by the ser-6 signaling cascade, which ultimately promotes lipolysis around fat storage.[49] These studies suggest that peripheral 5-HT may affect host metabolism in a manner independent of central effects.

Recent studies have targeted 5-HTRs, which have been reported to ameliorate insulin resistance by inhibiting 5-HT signaling in adipose tissue and thereby reducing lipolysis in visceral adipocytes. Treatment of HFD-fed mice with an antagonist of 5-HTR2B was found to improve insulin resistance, visceral adipose tissue inflammation, and hepatic steatosis.[50] Increased 5-HTR2B expression was also found in white adipose tissue of obese individuals and was positively correlated with metabolic parameters. In addition, there are also studies on 5-HTR6, which are targeted to develop a new generation of safe and more effective anti-obesity drugs.[51] The gut microbiota, as an important factor affecting 5-HT signaling, may also be used as a target to develop biological oral agents for the treatment of obesity in the future.

Colitis and other inflammatory related diseases

Increasing evidence suggests that immune cells in the peripheral system, including macrophages, T cells, and dendritic cells, may be involved in the production, storage, and function of 5-HT.[52] Aune *et al.*[53] found that inhibiting the synthesis of 5-HT could inhibit the proliferation of human T cells stimulated by interleukin-2 (IL-2), while adding 5-HTP, a precursor of 5-HT, could reverse the inhibitory effect on T cell proliferation. In addition, 5-HTR1A receptor antagonists also reduced T cell cytokine production *in vitro* in mouse models, thereby reducing cell-mediated immunity and *in vivo*

contact sensitivity responses. Similar results were found in the study of the 5-HTR1B receptor, where Yin *et al.*[54] used an antagonist of the 5-HT1B receptor to inhibit the proliferation of human and mouse primary helper T cells and human helper T cell lines. The 5-HT2A receptor was found to be an immune activator. When treated with a 5-HT2A receptor agonist and depleted of 5-HT in T cells of C57BL/6 mice, the production of IL-2 and interferon-γ (IFN-γ) in T cells was enhanced.[55] Not only does the 5-HT receptor exist on the surface of T cells, but the SERT responsible for transport is also expressed. Studies using the selective 5-HT reuptake inhibitor fluoxetine in rats have found that it can significantly increase the expression of the 5-HT transporter and the number of lymphocytes and may lead to a significant reduction in the concentration of cyclic adenosine monophosphate (cAMP) in lymphocytes and inhibit T cell activation and proliferation.[56] Both human and mouse T cells have the potential to store, degrade and synthesize 5-HT. O'Connell *et al.*[57] suggested that at the site of inflammation, activated T cells first release 5-HT, and dendritic cells can absorb, store and retransport 5-HT to naive T cells, thereby regulating the proliferation and differentiation of T cells. The involvement of T cells in the development of intestinal inflammation is also mediated by 5-HT. In inflammatory bowel disease (IBD), Th1 and Th17 helper cells often overreact. When TpH1 is knocked down in colitis mice, the secretion of inflammatory factors by dendritic cells decreases, and the levels of the proinflammatory cytokines IL-17 and IFN-γ secreted by T cells also decrease.[58] When 5-HT is supplemented, both the inflammatory response and disease progression are promoted, indicating that 5-HT plays an important role in the pathogenesis of colitis. In addition to TpH1 knockout mice, Ghia *et al.* also used a 5-HT synthesis inhibitor to construct an experimental colitis model of para-chlorophenylalanine (PCPA) and found that 5-HT stimulated macrophages in mice to produce pro-inflammatory cytokines, a process that was inhibited by inhibitors of nuclear factor κB.[59] The 5-HT precursor, 5-HTP, restores 5-HT levels and exacerbates colitis in TpH1 knockout mice. In addition, the synthetic food colorant Allura Red AC (ARAC) has been found to induce colitis through 5-HT in the colon.[60]

5-HT also plays an important role in other types of immune cells, such as macrophages, basophils, monocytes, and mast cells. Durk *et al.*[61] found mRNA expression of *5-HTR1E*, *5-HTR2A*, *5-HTR3*, *5-HTR4*, and *5-HTR7* in monocytes by reverse transcription and polymerase chain reaction (PCR). Among them, 5-HTR3, 5-HTR4, and 5-HTR7 are the main receptors of 5-HT for monocytes, which can activate and regulate the release of different chemokines and inflammatory factors. In macrophages, 5-HT can inhibit the release of inflammatory factors induced by LPS and upregulate M2

polarization-related genes such as *SERPINB2*, *THBS1*, *STAB1*, and *COL23A1*. The expression of M1-related genes such as *INHBA*, *CCR2*, *MMP12*, *SERPINE1*, *CD1B* and *ALDH1A2* was decreased.[62] In addition, 5-HTR2B and 5-HTR7 are involved in the maintenance of the anti-inflammatory state of macrophages. Mast cells can produce 5-HT by expressing TpH1,[63] and the expression level of TpH1 is higher than that of other immune cells.[64] Kushnir-Sukhov *et al.*[62] also found that mast cells express multiple 5-HTRs, including 5-HTR1A, 5-HTR1B, 5-HTR1E, 5-HTR2A, and 5-HTR2B and identified 5-HTR1A as the main receptor mediating the effect of 5-HT on mast cells. When the *5-HTR1A* gene is knocked down, the accumulation of mast cells (MCs) induced by 5-HT in the mouse dermis is inhibited. The relationship between gut microbiota and the peripheral immune system is complex and important. Not only 5-HT but also intestinal bacteria play a role in regulating inflammation-related factors by producing SCFAs, trimethylamine N-oxide, and reactive oxygen species.[65] Therefore, a better understanding of the functional mechanisms of the gut microbiota in various immune responses is necessary for determining the development of diseases.

Central nervous system diseases

In the CNS, most serotonergic neurons are located in the nuclei of the median raphe, including the descending medullary neurons of the raphe obscurus, magnus, pallidus, and their ascending pontine and mesencephalic counterparts in the raphe pontis, centralis, and dorsalis. Descending projections of the raphe nucleus affect spinal cord and brain stem mechanisms, which play an important role in central regulation of pain and pathological pain syndromes, in many cases originating from the gut. Ascending raphe projections point to higher integrated functional areas and are associated with advanced nervous function, including the regulation of mood, sleep, sex, and appetite.[12]

In addition, since 5-HT is impermeable across the blood-brain barrier, central serotonergic neurons are separated from peripheral serotonergic neurons, platelets, and enterochromaffin cells. It is believed that enterochromaffin cell-neural junctions are very different from neuromuscular junctions or any other synapse in the central or peripheral nervous system. 5-HT secreted by enterochromaffin cells acts locally in a paracrine manner. Intestinal serotonergic neurons are considered descending interneurons that innervate follower cells in both plexuses, and they do not project to smooth muscle or other effectors. Enterochromaffin cells are moving like the rest of the gastrointestinal epithelium, and nerves are not good at innervating moving targets. Therefore, conventional synapses cannot be found on them. In addition, enterochromaffin cells cannot focus their released transmitters as precisely as neurons.

Actually, the high secretion of 5-HT by enterochromaffin cells may compensate for the lack of close enterochromaffin cell-neuron connections. Thus, the concentration of local 5-HT in the mucosa is quite high, suggesting that the function of mucosal 5-HT is unlikely to be limited to nerve stimulation. For example, 5-HT is thought to affect the secretion and proliferation of crypt epithelial cells.[12]

In the gut, 5-HT activates both intrinsic and extrinsic PANs to regulate various physiological processes, including inflammatory responses, intrinsic peristaltic and secretory reflexes, and activation of extrinsic sensory nerves that transmit information to the brain, leading to the sensation of nausea and discomfort in the sensory CNS.[12,66] Cirrito *et al.* also found that 5-HT signaling may have the ability to alter Aβ levels and plaques in the brain. They gave mice SSRI and found that Aβ levels in the interstitial fluid were reduced by 25%. Direct injection of 5-HT into the hippocampus also reduced Aβ levels in the interstitial fluid.[67] A clinical cohort study of patients with PD has also shown an association between 5-HT and tremor behavior in patients with PD. Loss of serotonin transporters in the brain stem may aggravate the severity of tremor.[68] Based on this result, drugs that regulate 5-HT signal should be investigated in the treatment of Parkinson's related tremor. Among the receptors, 5-HTR3 and 5-HTR4 are closely related to functional gastrointestinal disorders.[69] Electrophysiological and anatomical studies have shown that 5-HTR4 is located in nerve terminals throughout the enteric nervous system (ENS) and mediates excitatory synaptic transmission. Activation of these presynaptic 5-HTR4 leads to an increase in the number of transmitters, such as acetylcholine, released from these terminals, which can enhance the reflex response.[70–72]

In IBS, inhibition of 5-HTR3 located on intrinsic sensory neurons reduces motor and secretory reflex activity and reduces activation of extrinsic sensory neurons that transmit signals to the CNS, suppressing sensory signals that cause pain and discomfort.[73] In rotavirus-induced diarrhea, 5-HTR3 has been shown to play a role by influencing intestinal motility, sensory neuron activation, cytokine secretion, and inflammatory responses, resulting in a reduced number of diarrhea days and diarrhea mouse counts in 5-HT3 receptor knockout mice compared with wild-type mice. However, vagal signaling to the vomiting center also occurs in the absence of 5-HTR3, indicating a complicated interaction between intestinal 5-HT and neural systems in the gut.[69,74,75]

Depression

Depression is a major cause of disability worldwide and is a heterogeneous suite of states characterized by sad mood and anhedonia (an inability to experience

pleasure). Currently, depression is generally considered a systemic disorder that can be triggered by different factors that ultimately target the 5-HT system in vulnerable individuals.[76–78] The therapeutic agents for treating depression are mainly antidepressants, most of which are SSRIs or serotonin-norepinephrine reuptake inhibitors (SNRIs). These agents are thought to act in part by increasing synaptic levels of monoamines, primarily 5-HT and norepinephrine, and by subsequent activation of serotonergic and noradrenergic postsynaptic receptors and autoreceptors. The therapeutic benefits of increased levels of monoamines were discovered through the efficacy of MAO inhibitors and tricyclic antidepressants in treating depression. However, antidepressants are only partially effective in moderate and more severe adult depression, suggesting that the monoamine hypothesis only partially explains depression. Although the idea that a single neurochemical causes depression is now considered simplistic, the low 5-HT hypothesis (also referred to as the "monoamine hypothesis") still lies at the foundation of most research on depression.[79,80]

The bidirectional crosstalk between the CNS, ENS, and gastrointestinal tract is referred to as the gut-brain axis (GBA), which connects the emotional and cognitive centers of the brain with the peripheral functions of the gastrointestinal tract. Accumulating evidence supports the theory that the gut microbiota plays an important role in the modulation of the GBA by directly or indirectly affecting the production of metabolites.[66,78–80] 5-HT is a key element of this axis and acts as a neurotransmitter in the CNS and ENS in the intestinal wall. Recent studies have shown that the gut microbiome or microbial species may affect the onset of depressive disorder by modulating neurotransmitters such as γ-aminobutyric acid (GABA) and 5-HT.

Using genome-wide shotgun metagenomics and untargeted metabolomics approaches, Yang *et al.*[81] identified significant differences in the abundance of gut microbiota between patients with major depressive disorder (MDD) and HCs. Patients with MDD were mainly characterized by increased abundance of the genus *Bacteroides* and decreased abundance of the genera *Blautia* and *Eubacterium*. Disrupted microbial genes and fecal metabolites were consistently mapped to amino acid (γ-aminobutyrate, phenylalanine, and tryptophan) metabolism. Therefore, a balance needs to be maintained between bacterial utilization of tryptophan and the tryptophan necessary for 5-HT synthesis in both enteric and CNS.

Given the role of 5-HT/tryptophan influenced by bacteria in the progression of depression disorder, manipulation of the gut microbiome can make a difference. In line with this notion, GF mice robustly and reproducibly display less anxiety-like or depression-like behaviors than their conventionally colonized counterparts,[82,83] with hippocampal concentrations of 5-HT and its major metabolite, 5-HIAA, significantly elevated in male GF animals,[84] while the anxious state can be reinstated with the introduction of a microbiota prior to critical time windows. Interestingly, although the peripheral availability of tryptophan returned to baseline values, colonization of GF animals after weaning was not sufficient to reverse the CNS neurochemical consequences of early deletion of microbiota in adulthood. Moreover, circulating levels of tryptophan decreased when these animals were introduced tryptophan metabolism-producing bacteria, such as *B. infantis*, into their gut.[70] Another study found that the gut microbiota from MDD patients transmits depressive behavior to AntiBiotics (ABX)-treated mice. Specifically, *B. uniformis*, *B. fragilis*, and *B. caccae* can impair hippocampal neurogenesis and reduce hippocampal 5-HT levels.[85] These results show that CNS neural conduction may be severely disturbed by the lack of normal gut microbiota.

In addition to probiotics that directly restore certain microbiota to retain the level of 5-HT,[85,86] drugs and prebiotics for interfering with 5-HT metabolism in the gut microbiota can also make sense. For example, Morinda officinalis oligosaccharides (MOOs), an oral drug approved in China for the treatment of depression, have been found to alleviate depression by increasing 5-HTP in the gut microbiota. Specifically, MOO increased tryptophan hydroxylase levels and inhibited 5-HTP decarboxylase activity in the gut microbiota, which accelerated 5-HTP production from tryptophan and reduced 5-HT generation, thus leading to the accumulation of 5-HTP. The increased 5-HTP from the gut microbiota was then absorbed to the blood, passed across the blood-brain barrier, and improved 5-HT levels in the brain.[87]

CONCLUSION

The mechanism of 5-HT action in the nervous system has been relatively well studied, and targeted inhibitors of the reuptake process are also used as drugs to treat patients with depression. Although the 5-HT produced in the gut cannot directly act on the brain through the blood-brain barrier, it plays a role in immune inflammation, obesity, IBS, and other aspects through a variety of 5-HTRs expressed in the intestinal tissue. With the understanding of the link between gut microbiota and 5-HT, gut bacteria are considered to be a key link in 5-HT signaling. It has also been found that the use of antidepressants targeting the 5-HT signaling pathway alters the composition of the gut microbiota, decreasing richness and increasing β diversity. However, the mechanisms by which antidepressants affect the microbiota remain

elusive. Possible mechanisms include inhibitors acting as efflux pumps affecting quorum sensing in the microbiota community.[88]

Although a number of studies have systematically explained that gut microbiota affects 5-HT in the occurrence and development of various diseases, the specific molecular mechanism behind it remains to be studied. For example, which bacteria play the most important role in indirectly or even directly producing 5-HT, what enzymes are involved, and whether the key enzymes can be modified? In addition, with the continuous development of technology, more and more methods have been used to study gut microbiota. The cell wall of bacteria can be labeled by D-amino acids with fluorescence to observe the three-dimensional imaging of gastrointestinal bacteria in mice.[89] Unlike ABX, phage has host specificity, so researchers have made use of phage specific knockout bacteria, which can provide effective help to study the role of a certain type of bacteria in gut microbiota.[90] Furthermore, in the process of studying intestinal bacteria, how to make the bacteria better colonize the stomach is also an urgent problem to be solved. By using biological orthogonality, molecules capable of biological orthogonality reaction can be added to the surface of the bacteria to be intragastric and the original colonizing bacteria in the host intestinal tract respectively, so that the bacteria can better colonize.[91]

The relationship between the gut microbiota and host is complex and changeable, and a series of factors, such as diet, daily schedule, and emotional changes, can be sensitively captured and responded to by the gut microbiota. Whether it is 5-HT or SCFAs, the current understanding is still insufficient to regard gut microbiota as a separate means to treat related diseases, and more is to make probiotics for adjuvant treatment. It is very important to determine more possible mechanisms and bacterial species that play a dominant role in the corresponding regulatory system. At the same time, the use of some relatively simple artificial bacterial communities may help people understand the relationship between different bacterial species to better facilitate the regulation of their composition. Diseases such as colitis and IBS, which currently seriously affect the quality of life of patients, may be a breakthrough in the future because of the study of gut microbiota. An increasing number of researchers use more standardized and rigorous quantitative indicators and technical methods to explore the causal relationship between gut microbiota and host metabolism. It is believed that such strategies will greatly promote the development of gut microbiota.

DECLARATION

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Parts of the figure were drawn using pictures from scidraw.io (upload by Chilton, 2020) and Servier Medical Art.

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Author contributions
Hu GF: Conceptualization, Investigation, Writing—Original draft. Zhu YJ: Data curation, Formal analysis. Ding SS: Methodology, Writing—Review and Editing. Zheng LM: Supervision, Project administration, Funding acquisition.

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Lemin Zheng is the Editor-in-Chief of the journal. The article was subject to the journal's standard procedures, with peer review handled independently of the Editor-in-Chief and the affiliated research groups.

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