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Review article

Progress in the study of intestinal microbiota involved in morphine tolerance

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ABSTRACT

Morphine is a widely used opioid for treatment of pain. The attendant problems including morphine tolerance and morphine dependence pose a major public health challenge. In recent years, there has been increasing interest in the gastrointestinal microbiota in many physiological and pathophysiological processes. The connectivity network between the gut microbiota and the brain is involved in multiple biological systems, and bidirectional communication between them is critical in gastrointestinal tract homeostasis, the central nervous system, and the microbial system. Many research have previously shown that morphine has a variety of effects on the gastrointestinal tract, but none have determined the function of intestinal microbiota in morphine tolerance. This study reviewed the mechanisms of morphine tolerance from the perspective of dysregulation of microbiota-gut-brain axis homeostasis, by summarizing the possible mechanisms originating from the gut that may affect morphine tolerance and the improvement of morphine tolerance through the gut microbiota.

1. Introduction

Chronic pain is an important complaint of many clinical diseases, seriously hampering patients' physical and mental health, which has become a global public health problem [1]. Over the past three decades, the incidence of chronic pain has been increasing every year, and this has been accompanied by a rapid abuse of opioids [2]. At least 3% of adult patients who receive long-term treatment with opioid prescription drugs for non-cancer pain (neuropathic pain, chronic musculoskeletal pain, chronic back pain.) are reported to use them irrationally for non-therapeutic purposes [3]. Adolescents take opioid prescription medicines irrationally for pain alleviation and mental relaxation [4]. Drug tolerance can lead to an increase in opioid medication dosage, leading to serious side effects (such as drug dependence, overdose, and death) As a result, the U.S. made the "opioid crisis" a national public health emergency in 2017 [5]. In 2020 alone, the Centers for Disease Control and Prevention reported more than 100,000 deaths from opioid overdoses [6]. As a result, addressing these concerns is critical. Opioid receptors are G protein-coupled receptors, mainly classified as μ receptors

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(MOR), δ receptors (DOR), and κ receptors (KOR). Morphine and the other more commonly used opioids mostly act at MOR [7]. Opioids have effects on gastrointestinal function, including prolonged intestinal motility, delayed gastric emptying, increased myogenic tone, decreased peristalsis, and constipation, which are collectively referred to clinically as "Nacrotic Bowel Syndrome" [8,9]. Morphine, the prototype of opioids, is currently the most effective and widely used analgesic in the clinical treatment of chronic pain. The 2016 Polyanalgesic Consensus Conference (PACC) guidelines recommended morphine as firstline intrathecal monotherapy for localized and diffuse chronic pain of cancer-related and non-cancer-related etiologies [10]. Morphine is also frequently used to treat persistent pain caused by neurological diseases. Many clinical trials have also had clinical success with the use of oral morphine for cancer pain. Morphine has a significant analgesic effect on pain, but it also has an effect on a person's mental and emotional state. Studies have shown that morphine has some degree of sedative and anxiety-relieving effects. However, the development of tolerance, dependence, and affective signs of withdrawal following repeated or prolonged administration limits its therapeutic utility. In recent years, morphine tolerance has also led to a continuously deteriorating in morphine consumption, which should be a cause for alarm [11]. Therefore, exploring effective methods to relieve morphine tolerance is an important means to increase the analgesic effect of morphine and reduce morphine dosage. Previous studies on morphine tolerance have focused on the central nervous system. Morphine tolerance is associated with the downregulation of opioid receptors, altered neuronal plasticity due to sustained stimulation of G protein decoupling, and increased synthesis of anti-opioid substances [12,13]. However, current knowledge of the central nervous system does not explain the phenomenon of morphine tolerance well. In clinical practice, some drugs targeting the central nervous system in combination with morphine fail to reduce the development of morphine tolerance. In this paper, we look at the relationship between the gut microbiota and morphine tolerance, with a focus on the microbiota-gut-brain axis.

2. The importance of the intestinal microbiota

The intestinal microbiota is the most complex and abundant micro-ecosystem in the body [14], and its homeostatic balance is essential to maintain the health of the body [15]. Dysregulation of intestinal microbiota homeostasis will lead to many diseases, such as obesity, diabetes, cardiovascular diseases, and neurological diseases [16–18]. Also, intestinal microbiota plays an important role in the host's immune system, inflammation, and cancer prevention [19–21]. In humans and animals, gut microbiota also plays an important role in regulating brain function, mood, stress, and behavioral responses to rewards [22–25]. In addition, intestinal microbiota has an impact on various neuropsychiatric disorders (Parkinson's disease, depression, anxiety, autism, etc.) and can further influence host behavior and performance by affecting gene expression and neurotransmitter metabolism [26,27] (Fig. 1). All of the above studies suggest that intestinal microbiota and the neuropsychiatric system are closely related. Although it is unclear whether these examples are driven by direct gut-brain interactions or mediated by other factors induced by the disease state. Further investigation is necessary

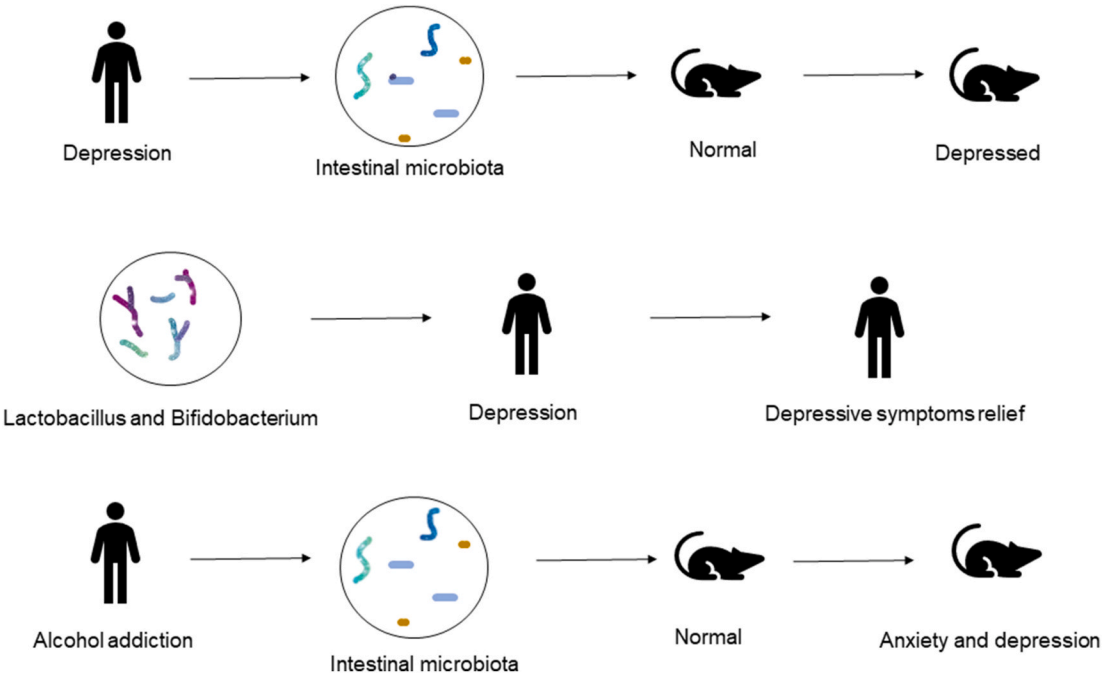

Fig. 1. Intestinal microbiota and neuropsychiatric disorders are closely linked. The transplantation of fecal microbiota from depressed patients to rats induces a depression-like phenotype in rats. A combination of Lactobacillus and Bifidobacterium can effectively improve depression in patients with severe depression. Transplantation of fecal microbiota from alcoholic patients into healthy mice shows symptoms associated with depression and anxiety. The humanoid icons represent humans and the animal icons represent rats and mice. The arrows in the figure represent evolutionary.

to fully understand the possible bidirectional interactions that exist between the brain, gastrointestinal tract, and microbiome.

3. Microbiota-gut-brain axis

Numerous studies have been done to look into the connection between neurological, behavioral, or inflammatory problems and the gut-brain axis [28,29]. The intestinal microbiota has a bi-directional regulatory effect on the "gut-brain axis" through neural, immune, and endocrine channels, thus establishing a new concept of the "microbiota-gut-brain axis" [30]. It involves a network of connections between multiple biological systems, allowing bidirectional communication between gut bacteria and the brain, and is critical in maintaining homeostasis in the gastrointestinal tract, central nervous system, and microbial system, while its imbalance in functional homeostasis plays an important role in a variety of neurological disorders [31,32]. The microbiota-intestine-brain axis consists of 3 main interactions pathways: (1) Endocrine signals: the microbiota can influence the neuroendocrine system via metabolites such as short-chain fatty acids (SCFAS) [33], which modulate neurotransmitter concentrations such as -aminobutyric acid (GABA) and 5-hydroxytryptamine (5-HT). These neurotransmitters have an impact on the neurological system, either directly or indirectly [34,35].. Gut microorganisms can affect their host's appetite and feeding behaviours by modulating production of endocrine signals from enteroendocrine cells (EECs) in the gut epithelium, including production of the hormone glucagon-like peptide 1 (GLP1) [36]. (2) Neural pathway: The neural pathway physically connects the gut and the brain, and the microbiota and its metabolites can act on the vagus nerve and the enteric nervous system (ENS) to affect the brain and behavior. Vagus nerve efferent fibres propagate information from the brain to the viscera and have an important role in immune function and metabolism. These factors, in turn, may alter the gut microbiota by affecting the environment of the gut, which implicates the vagus nerve as an important mediator of bidirectional communication both to and from the brain [37]; (3) Immune system: Both the central nervous system and the gut microbiota directly affect, and are affected by, the immune system. Intestinal microbiota is a key factor in regulating the development and function of the peripheral immune system, in which microglia and inflammatory cytokines play a key mediating role [38] (Fig. 2). Notably, because of the involvement of the gut-brain axis, approaches for modifying the gut microbiota can also be used to treat central nervous system illnesses. Gut microbiota depletion by an oral antibiotic cocktail of ampicillin, metronidazole, neomycin, and vancomycin (AMNV) significantly reduced the incidence of intracranial aneurysm via decreasing macrophage infiltration and the expression of pro-inflammatory cytokines such as IL-1β and IL-6 in vascular wells [39]. Additionally, Lactulose supplementation was shown to significantly improve the functional outcome of stroke, which is possibly mediated by repairing intestinal barrier injury and improving gut microbiota dysbiosis following stroke [40]. In the following, we discuss some important studies analyzing the effects of morphine tolerance on the brain through the microbiota.

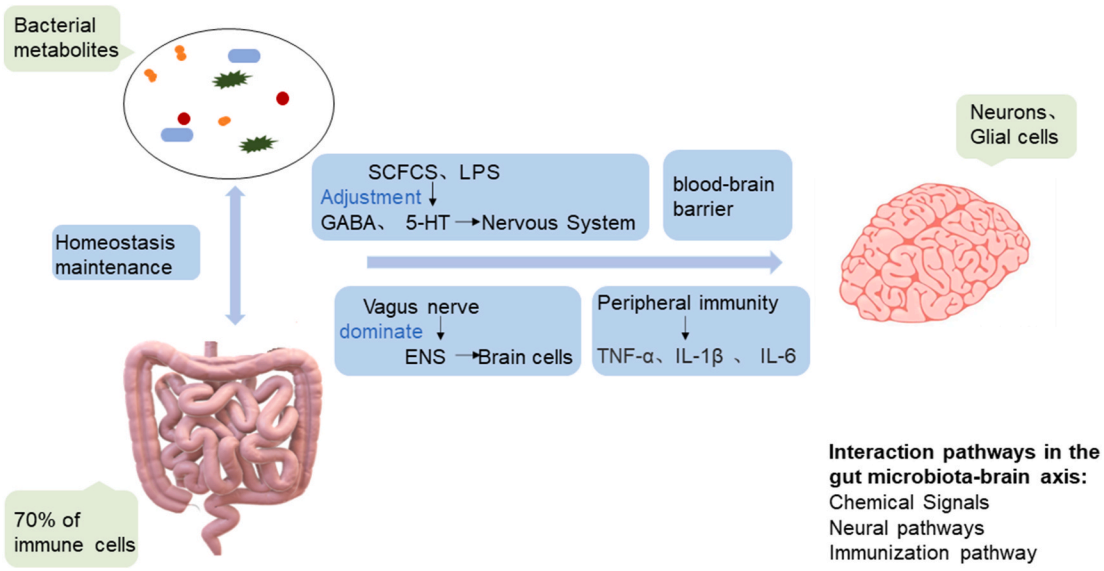

Fig. 2. Interaction pathways in the microbiota-gut-brain axis. The microbiota can influence the neuroendocrine system via metabolites such as short-chain fatty acids (SCFAS), which modulate neurotransmitter concentrations such as -aminobutyric acid (GABA) and 5-hydroxytryptamine (5-HT). These neurotransmitters have an impact on the neurological system, either directly or indirectly. The microbiota and its metabolites can act on the vagus nerve and the enteric nervous system (ENS) to affect the brain and behavior. Intestinal microbiota is a key factor in regulating the development and function of the peripheral immune system, in which microglia and inflammatory cytokines play a key mediating role. SCFCS: short-chain fatty acids, LPS: lipopolysaccharide, GABA: γ-aminobutyric acid, 5-HT: 5-hydroxytryptamine, ENS: Enteric Nervous System, TNF-α: tumor necrosis factor α, IL-1β: Interleukin 1β, IL-6: Interleukin 6.

4. Morphine affects the intestinal microbiota

Under normal conditions, the intestine of humans and mice is colonized mainly by anaphylaxis and thick-walled bacteria, with several other phyla present in smaller proportions, but alterations in bacterial composition as well as localization play an important role in pathogenesis. Significant reductions in pseudobacillus and thick-walled bacteria were found in the feces of mice chronically injected with morphine [41], it also reduced the ratio of pseudobacillus to thick-walled bacteria [42], which was associated with an increase in systemic inflammation, making mice more likely to develop enteritis [43,44]. The relative abundance of pathogenic bacteria, such as Flavobacterium, Enterococcus, Clostridium, and Clostridium tumefacient, was also found to be increased in mouse fecal samples [45]. In addition, morphine induces an increase in gram-positive bacteria, while disrupting bile acid metabolism, further affecting intestinal health through the hepatic-intestinal cycle [42]. Significant changes in the composition and variety of the intestinal flora were also discovered in cirrhotic individuals treated with morphine for chronic pain for 5 months [46]. Reduced intestinal epithelial cell proliferation and abnormal morphology, accompanied by disruption of small intestinal barrier integrity, were also observed in morphine-treated AIDS patients [47].

As a complex organ, intestinal microbiota imbalance is also closely related to intestinal barrier disruption and bacterial translocation [48]. Once the intestinal microbiota is dysbiosis, the intestinal mucosal barrier is easily damaged, making the intestinal permeability increase, which can induce bacterial translocation and lead to mild or severe systemic inflammatory reactions and even multi-organ dysfunction [49]. Morphine causes not only the changes in intestinal microbiota stated above, but also aberrant intestinal shape and decreased epithelial cell proliferation, ultimately leading to breakdown of the patient's intestinal barrier [47]. This is closely related to Toll-like receptors (TLRs), which recognize a variety of microbial components and play a central role in host-microbiota interactions [50]. Long-term morphine use induces significant increases in TLR2 and TLR4 expression in intestinal and systemic immune cells, leading to further elevations in the pro-inflammatory cytokines Interleukin 6 (IL-6) and tumor necrosis factor α(TNF-α). In turn, proinflammatory cytokines exacerbate intestinal microbiota imbalances, leading to impaired intestinal integrity and bacterial translocation, thereby increasing inflammation and morphine tolerance [51,52]. Altered intestinal microbes may impact morphine's enterohepatic recirculation and consequently its analgesic efficacy. Also, TLRs activation can lead to decreased expression of tight junction proteins on intestinal epithelial cells, which can further lead to disruption of the intestinal barrier.

Alterations in the gut microbiome frequently occur together with disturbances in enteric neural control in pathophysiological conditions [53]. Disruptions in the gut microbiota may have long-term implications on ENS development. Changes in the gut microbiota have been shown in studies to modify the ENS, impair barrier function, and cause a number of disorders such as irritable bowel syndrome, inflammatory bowel disease, and neurodegeneration [54]. In addition to the changes in the intestinal microbiota described above, morphine also has an effect on the enteric nervous system, Electrophysiological recordings of enteric neurons show a general inhibitory effect of morphine on the excitability of enteric neurons [55,56]. Morphine inhibits the formation of action potentials required for neurotransmitter release, and the fundamental effect of morphine on gastrointestinal motility and secretion is the suppression of neuron excitability. In addition, morphine also induces a decrease in intestinal peristaltic frequency and a delay in intestinal contents transport in mice through the activation of central and peripheral opioid receptors [57]. Enteric glial cells are the most numerous cell type in the enteric nervous system and are a key factor in regulating the function of the gastrointestinal tract by producing trophic factors, and morphine is also capable of causing enteric glial cell dysfunction [57]. All of the above evidence suggests that morphine not only causes an imbalance in intestinal microbiota but is also strongly associated with intestinal barrier disruption and decreased intestinal function [48,58,59].

5. Involvement of gut microbiota in morphine tolerance

Researchers have begun to wonder whether the gut microbiota plays a role in morphine tolerance because of the extensive effects of morphine on the gut. Further studies found that beneficial bacteria such as Lactobacillus and Bifidobacterium were significantly reduced in morphine-tolerant mice. In fact, at the intestinal level, beneficial bacteria (such as Lactobacillus and Bifidobacterium) have a significant protective effect on intestinal epithelial cell integrity [60]. Their reduction disrupts intestinal integrity, promotes bacterial translocation, and exacerbates inflammation. Similarly, after 8 days of treatment with incremental doses of morphine, mice treated with broad-spectrum antibiotics had considerably decreased analgesic tolerance in the tail-flick and hot-plate tests [41]. Despite the fact that the mechanisms involved are unknown, it is obvious that decreasing gut flora decreases the development of morphine tolerance. In this study, however, the reduced gut microbiota prevented morphine tolerance-induced increases in intestinal permeability, colonic mucosal degradation, and colonic Interleukin 1 (IL-1) expression. Also to determine which components of the antibiotic mixture had the greatest effect on preventing tolerance, mice were treated with various combinations of antibiotics, and it was found that the combination containing vancomycin had the best effect. Although vancomycin alone was successful in preventing tolerance, it was less effective than a combination of streptomycin and ampicillin [41]. Other research has found that depletion of intestinal de-conjugating bacteria linked with the deglucuronidation of morphine-3-glucuronide, the main metabolite of morphine, leads to decreased enterohepatic circulation of morphine, which results in lower systemic morphine levels over time [61]. It is also plausible that morphine-induced microbial dysbiosis may result in altered morphine metabolism, thus contributing to morphine tolerance.

6. Development of morphine tolerance

The establishment and maintenance of morphine tolerance is a vicious cycle, and its principal effect on the body is mediated by MOR. Previously, it was widely assumed that morphine's analgesic mechanism was the drug's direct influence on the central nervous

system, and so many investigations on morphine tolerance and dependency focused on the central nervous system [62–64]. It was discovered that morphine-tolerant animals had higher astrocyte activity in their spinal cord and hippocampus, but inhibiting astrocyte activity could considerably reduce morphine tolerance [65]. At the same time, long-term high doses of morphine cause significant microglia activation [66,67]. Microglia activation releases pro-inflammatory cytokines and chemokines, such as TNF-α, IL-1β, IL-6, CXCL1, which cause hyperexcitability of nociceptive neurons and lead to the development of morphine tolerance [68,69]. However, the current knowledge of neurons does not provide a good explanation for some of the phenomena of morphine tolerance. For example, the transplantation of brain extracts from rats in a morphine tolerance model into mouse brain tissue failed to induce morphine tolerance, implying that the primary mechanism of morphine tolerance may not reside in the higher centers [70]. In contrast, when combined with morphine, the selective peripheral opioid receptor antagonist methylnaltrexone bromide has no effect on the analgesic efficacy of morphine but does delay the development of morphine tolerance. Corder et al. recently demonstrated that analgesic tolerance to chronic morphine was also lost following conditional deletion of MOR in primary neurons, implying that peripheral primary neurons are required for morphine tolerance [71].

7. Gut microbiota modulates dorsal root ganglion inflammatory response mediating morphine tolerance

The dorsal root ganglion (DRG), as the first level of nociceptive afferent neurons, also plays an extremely important role in the peripheral mechanisms of nociception [72]. In recent years, it has been discovered that morphine-induced analgesia is still present after MOR in DRG injury receptors is knocked down, indicating that peripheral DRGs are involved in mediating morphine tolerance [71]. Previously, monocyte chemotactic protein 1 (MCP-1) in the spinal cord was found to be involved in the development of morphine tolerance, while MCP-1 has a significant chemotactic effect on macrophages [73]. MCP-1 was also discovered to be significantly elevated in the DRG of morphine-tolerant mice, pointing to increased DRG excitability brought on by macrophage-induced neuroinflammation as one of the key mechanisms of morphine tolerance. This finding also supports the critical function of peripheral DRG neurons in morphine tolerance.

Additionally, the removal of intestinal microbiota with antibiotics reduced the rise in morphine-induced inflammatory factors in the DRG, suggesting that intestinal microbiota may play a role in morphine-induced macrophage infiltration, neuroinflammatory responses, and morphine tolerance (Fig. 3). According to other research, TTX-R Na channels play a role in how gastrointestinal microbiota influences the growth of morphine tolerance in nociceptive primary afferent neurons [74].

8. Gut microbiota is involved in the development of morphine tolerance by regulating TLR2 and TLR4

The mechanisms of morphine tolerance are complex, and immunity plays an important role in addition to the mechanisms of the

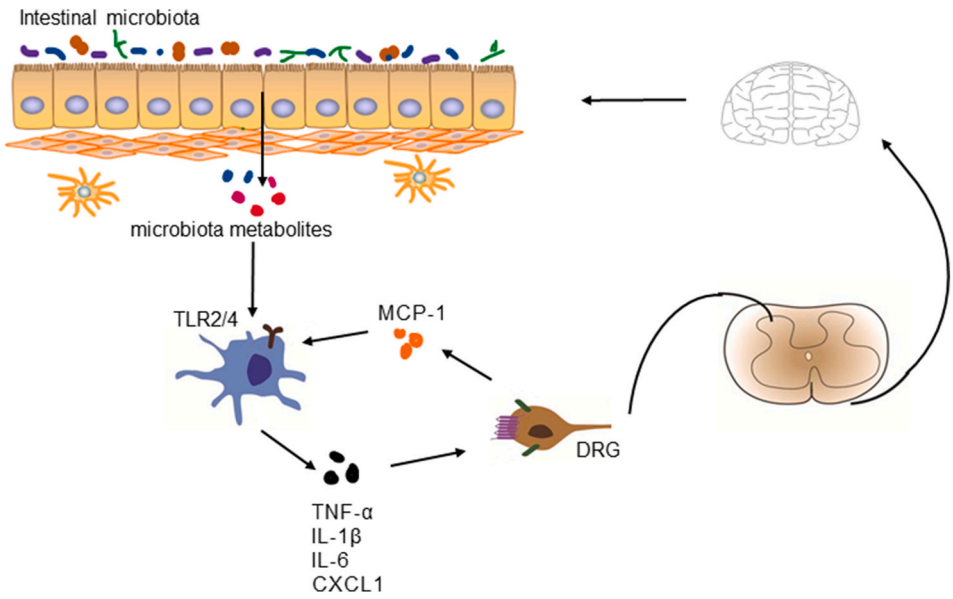

Fig. 3. Schematic representation of the mechanism by which intestinal microbiota modulates the dorsal root ganglion inflammatory response mediating morphine tolerance. Intestinal bacterial products activate peripheral macrophages via TLR2, which infiltrate and release MCP-1 and inflammatory mediators (TNF-α, IL-1β, IL-6, CXCL1) in the dorsal root ganglion, inducing dorsal root ganglion neuroinflammation, leading to increased excitability of the dorsal root ganglion, and ultimately leading to morphine tolerance. Increased levels of the pro-inflammatory cytokines IL-6 and TNF- in the gut aggravate gut microbiota imbalances, eventually leading to gut inflammation and morphine tolerance. It's a never-ending circle. MCP-1: Monocyte Chemoattractant Protein-1, TNF-α: tumor necrosis factor α, IL-1β: Interleukin 1β, IL-6: Interleukin 6, CXCL1: C-X-C motif chemokine ligand 1.

neurons themselves. It has been found that an imbalance in intestinal microbiota can lead to an overactive systemic immune system [75]. Morphine given in vivo suppresses a variety of immune responses that involve the major cell types in the immune system, including natural killer (NK) cells, T cells, B cells, macrophages and polymorphonuclear leukocytes (PMNs) [76]. The opioid receptor antagonist naloxone can entirely reverse morphine's inhibition of human T lymphocytes [77]. Further evidence subsequently emerged that opioids suppress the immune system at various stages, starting with innate immune cells, including antigen presentation, and ending with regulatory T lymphocyte activation and differentiation, and increase the risk of opportunistic infections [78–80]. TLRs, as one of the most representative receptors in the intrinsic immune response, mediate a series of important immune response reactions [81]. Activation of TLRs transmits activation signals and induces activated cells to express a series of immune molecules [82]. TLRs appear to play a key role in the onset and maintenance of pain, according to accumulating research [83,84]. It has been found that morphine can directly bind to TLR4 in microglia, producing an effect similar to that of lipopolysaccharide LPS, which in turn activates neuroinflammation to affect the analgesic effect of morphine [85]. Therefore, the TLR4 signaling pathway is thought to be the main mechanism of morphine tolerance and pain sensitivity. Another study found that the knockdown of the TLR2 gene can alleviate the development of morphine tolerance and inhibit microglia activation and inflammatory factor expression [86]. However, it is now established that morphine induces analgesic tolerance and increases the expression levels of TLR2 and TLR4 in intestinal epithelial cells and immune cells. Importantly, as mentioned above, morphine-induced changes in intestinal permeability and translocation of the intestinal microbiota are also dependent on TLR2 and TLR4 [87]. It is proposed that the activation of TLR2 and TLR4 by intestinal microbiota causes an imbalance in intestinal microecology, and that the ensuing pro-inflammatory cytokines may promote the establishment of morphine analgesic tolerance.

9. Involvement of the gut microbiota in the effects of morphine on the brain

Opioid peptides from food have been called exorphins. Excess exorphins have been linked to the development of autism spectrum disorder (ASD) in several studies. Opioid peptides derived from exorphins have been found in urine of autistic patients [88]. In children with ASD, the homeostasis of the intestinal microbiota was found to be compromised, accompanied by damage to the intestinal barrier [89]. Whereas the high permeability of the intestinal barrier in ASD patients is associated with high expression of various tight junction proteins (claudin-2, claudin-10, and claudin-15), these alterations promote the entry of exorphins into the bloodstream, leading to an opioid overload [90]. Exorphins can enter the brain and interact with the central nervous system due to the inflammatory milieu of the gut and the blood-brain barrier [91]. It was found that some bacteria of the genus Bifidobacterium, such as B. longum and B. bifidum, can degrade exorphins [92]. Long-term morphine use leads to a decrease in intestinal bifidobacteria, suggesting that the gut microbiota may affect the central nervous system by altering levels of exorphins. Significant changes in intestinal microbiota were also found in patients with schizophrenia, as well as significant intestinal inflammation and intestinal barrier damage [93]. Zhu et al. transplanted schizophrenic patients' fecal bacteria into mice, which likewise displayed schizophrenia-like symptoms [94]. This also strongly suggests that morphine can establish a close connection with the neuropsychiatric system by altering the intestinal microbiota.

SCFAS are lipids produced by intestinal microorganisms through fermentation of dietary fiber, which may act on the central nervous system by modulating neuroplasticity, gene expression, and the immune system [33]. After 5 months of morphine usage, the levels of SCFAS-producing microbiota in the body were drastically reduced in patients with chronic pain owing to cirrhosis, resulting to endotoxemia and elevated proinflammatory cytokine IL-6, which has an influence on the central nervous system [46]. Tryptophan (Trp) is an essential amino acid in the human body and is related to cognitive function [95]. In the brain, Trp is converted to 5-HT after crossing the blood-brain barrier. 5-HT concentration in the brain is associated with a variety of neurological activities, including motor function, emotion modulation, and cognition [96]. 5-Hydroxytryptamine is catalyzed by monoamine oxidase to 5-hydroxyindoleacetic acid and excreted in urine. The majority of Trp is metabolized in the intestine, while the remaining small portion enters the circulation and is metabolized in the central nervous system to produce important neurotransmitters [97]. Thus, intestinal microbiota can indirectly alter the function of the central nervous system by regulating the metabolism of Trp in the intestine, achieving a linkage between intestinal microbiota and the brain. Long-term morphine usage can raise Trp and 5-hydroxy indole acetic acid levels in the brain, which can have an influence on cognition [98]. Aside from cognitive impairments, the most serious concern of long-term opioid usage in chronic pain sufferers is the development of depression and anxiety symptoms. According to the monoaminergic hypothesis, depression is associated with homeostatic imbalances in the 5-HT and norepinephrine (NA) systems [99]. Dysregulation of the intestinal tight junction protein claudin-5 was found in depression, further leading to systemic inflammation as well as loss of blood-brain barrier integrity. At the same time, long-term morphine use leads to significant activation of microglia, which in turn releases the pro-inflammatory cytokines IL-1β, and IL-6 [66,67]. IL-1β and IL-6 inhibit 5-HT production, resulting in increased generation of quinolinic acid, which can overstimulate neurons involved with depression and induce brain damage [100]. Chronic morphine use for pain may also lead to obesity, the agonism of MOR could predispose the patient to the development of obesity [101]. Beta-casomorphin-7 (BCM-7) is an opioid peptide derived from milk. It may influence obesity via MOR pathways [102]. The simultaneous change of the blood-brain barrier and intestinal tight junctions allows opioid peptides derived from food to enter the brain, resulting in the destruction of glial and neuronal cells via local and systemic inflammation [103].

10. Improvement of morphine tolerance by gut microbiota

Drug tolerance is a worldwide issue that has yet to be solved. As an inhibitor of microglia activation, minocycline has previously been shown to slow the development of morphine tolerance [104]. However, the role of gut microbiota in understanding morphine tolerance processes is becoming clear. Simultaneously, many research including alteration of gut bacterial composition have

demonstrated that gut microbiota can intervene and reduce tolerance developed during long-term opioid use [41]. These findings have substantial therapeutic significance because they imply that modulating gut microbiota could be a potential strategy for the prevention and treatment of opioid analgesic tolerance. Broad-spectrum antibiotics may reduce morphine tolerance by reducing the gut microbiota. It also prevents chronic morphine-induced increase in intestinal permeability, colonic mucosal destruction and colonic IL-1β expression. For example, vancomycin mostly eliminates gram-positive bacteria from the colon and is most efficient in delaying morphine tolerance [41]. This raises the possibility that Gram-positive bacteria play a key part in the formation of morphine tolerance and provides ideas for further research. However, antibiotics' effects may be independent of their spectrum of activity, and each antibiotic may have a unique mechanism. Kang et al. demonstrated in their study that antibiotics prevented morphine-induced decrease in dorsal root ganglion excitability [41]. In recent years, the use of probiotics in mouse models and humans has also been increasing. Probiotics rich in bifidobacteria and lactobacilli not only restore morphine-induced intestinal microbiota disorders but also reduce morphine tolerance [105]. Probiotics VSL#3, for example, offers a mechanism for reducing morphine tolerance by replenishing some gut microbial components while also lowering levels of the pro-inflammatory cytokines IL-6, IL-1β, and TNF-α. Isorhynchophylline also reverses morphine-dependent changes in gut microbiota alpha and beta diversity, composition, and abundance in experiments in zebrafish [106]. It reduces the rise in the Bacteroidetes/Firmicutes ratio caused by morphine tolerance. Ginsenoside Rg1 has also been demonstrated to attenuate morphine dependence by regulation of gut microbiota and tryptophan metabolism [107]. Fecal microbiota transplantation as a strategy to restore microbial homeostasis is currently being studied in the context of opioid-induced ecological dysregulation [42].

11. Conclusion and outlook

Morphine, the most effective and widely used analgesic for chronic pain in clinical practice, can cause morphine tolerance with long-term use. Morphine tolerance also increases the risk of overdose and side effects (morphine dependence and drug overdose deaths, Nacrotic Bowel Syndrome, anxiety.), thus morphine tolerance greatly limits its clinical use and becomes a problematic issue in pain management. The intestinal microbiota is a diverse community that maintains a close relationship with its host. It does not just affect the gut, it affects multiple systems throughout the body. Morphine tolerance affects the gut and may be involved in various pathophysiologic processes in the central nervous system through the gut microbiota. The co-evolution of animals and their associated microbial communities appears to have resulted in complex biological communications between the gut and the brain, a fascinating perspective that requires more investigation.

Countering morphine tolerance from the perspective of the gut microbiota is an innovative idea. Whether broad-spectrum antibiotics, probiotics, or fecal transplants are highly desirable in morphine tolerance models. An important question that arises from the use of antibiotics is whether the loss of tolerance is due to gut bacterial depletion/alteration or other off-target effects. Probiotic therapy may be a promising, safe and inexpensive treatment to prolong the efficacy of morphine and attenuate analgesic tolerance. Given the multitude of biological systems involved in the gut microbiota-gut-brain axis, the mechanisms and pathways of gut microbiota action may act synergistically to modulate various aspects of morphine tolerance, and more research is still needed to understand it.

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Data availability statement

Data related to the study are not stored in publicly accessible repositories.

CRediT authorship contribution statement

Ke Bi: Writing – original draft. **Yi Lei:** Resources. **Deshenyue Kong:** Funding acquisition. **Yuansen Li:** Resources. **Xuan Fan:** Resources. **Xiao Luo:** Investigation. **Jiqun Yang:** Project administration. **Guangqing Wang:** Supervision. **Xuejun Li:** Data curation. **Yu Xu:** Writing – review & editing, Conceptualization. **Huayou Luo:** Writing – review & editing, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] A.I. Basbaum, et al., Cellular and molecular mechanisms of pain, Cell 139 (2) (2009) 267–284.
[2] G. de Guglielmo, et al., Editorial: opioids and opioid-use disorders, Front. Pharmacol. 14 (2023) 1227174.
[3] R. Kerry, et al., Spatial analysis of drug poisoning deaths in the American West, particularly Utah, Int. J. Drug Pol. 33 (2016) 44–55.
[4] B. Ali, et al., Trends in drug poisoning deaths among adolescents and young adults in the United States, 2006-2015, J. Stud. Alcohol Drugs 80 (2) (2019) 201–210.
[5] N.D. Volkow, F.S. Collins, The role of science in addressing the opioid crisis, N. Engl. J. Med. 377 (4) (2017) 391–394.
[6] J. Trecki, et al., Notes from the field: increased incidence of fentanyl-related deaths involving para-fluorofentanyl or metonitazene - Knox County, Tennessee, November 2020-August 2021, MMWR. Morbid. Mortality Weekly Rep. 71 (4) (2022) 153–155.
[7] C. Stein, Opioid receptors, Annu. Rev. Med. 67 (2016) 433–451.
[8] A.D. Farmer, et al., *Narcotic bowel syndrome.* The Lancet, Gastroenterol. Hepatol. 2 (5) (2017) 361–368.
[9] H.I. Akbarali, A. Inkisar, W.L. Dewey, Site and mechanism of morphine tolerance in the gastrointestinal tract, Neuro Gastroenterol. Motil.: Off. J. Eur. Gastrointest. Motility Soc. 26 (10) (2014) 1361–1367.
[10] T.R. Deer, et al., Intrathecal therapy for chronic pain: a review of morphine and Ziconotide as firstline options, Pain Med. 20 (4) (2019) 784–798.
[11] P. Skolnick, The opioid epidemic: crisis and solutions, Annu. Rev. Pharmacol. Toxicol. 58 (2018) 143–159.
[12] J.T. Williams, et al., Regulation of μ-opioid receptors: desensitization, phosphorylation, internalization, and tolerance, Pharmacol. Rev. 65 (1) (2013) 223–254.
[13] T.W. Vanderah, et al., Dynorphin promotes abnormal pain and spinal opioid antinociceptive tolerance, J. Neurosci.: Off. J. Soc. Neurosci. 20 (18) (2000) 7074–7079.
[14] A. Heintz-Buschart, P. Wilmes, Human gut microbiome: function matters, Trends Microbiol. 26 (7) (2018) 563–574.
[15] E.A. Mayer, K. Tillisch, A. Gupta, Gut/brain axis and the microbiota, J. Clin. Investig. 125 (3) (2015) 926–938.
[16] I. Sekirov, et al., Gut microbiota in health and disease, Physiol. Rev. 90 (3) (2010) 859–904.
[17] C.A. Lozupone, et al., Diversity, stability and resilience of the human gut microbiota, Nature 489 (7415) (2012) 220–230.
[18] H. Tilg, A. Kaser, Gut microbiome, obesity, and metabolic dysfunction, J. Clin. Investig. 121 (6) (2011) 2126–2132.
[19] C. Cremon, et al., Effect of CNCM I-1572 on symptoms, gut microbiota, short chain fatty acids, and immune activation in patients with irritable bowel syndrome: a pilot randomized clinical trial, United Eur. Gastroenterol. J. 6 (4) (2018) 604–613.
[20] S. Sivaprakasam, P.D. Prasad, N. Singh, Benefits of short-chain fatty acids and their receptors in inflammation and carcinogenesis, Pharmacol. Therapeut. 164 (2016) 144–151.
[21] J.K. Nicholson, et al., Host-gut microbiota metabolic interactions, Science (New York, N.Y.) 336 (6086) (2012) 1262–1267.
[22] S.M. Collins, P. Bercik, The relationship between intestinal microbiota and the central nervous system in normal gastrointestinal function and disease, Gastroenterology 136 (6) (2009) 2003–2014.
[23] R. Diaz Heijtz, et al., Normal gut microbiota modulates brain development and behavior, Proc. Natl. Acad. Sci. U.S.A. 108 (7) (2011) 3047–3052.
[24] G. Clarke, et al., The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner, Mol. Psychiatr. 18 (6) (2013) 666–673.
[25] S. Cussotto, et al., The neuroendocrinology of the microbiota-gut-brain Axis: a behavioural perspective, Front. Neuroendocrinol. 51 (2018).
[26] G. Sharon, et al., The central nervous system and the gut microbiome, Cell 167 (4) (2016) 915–932.
[27] Q. Li, J.M. Zhou, The microbiota-gut-brain axis and its potential therapeutic role in autism spectrum disorder, Neuroscience 324 (2016) 131–139.
[28] G.Z. Réus, et al., Imipramine reverses alterations in cytokines and BDNF levels induced by maternal deprivation in adult rats, Behav. Brain Res. 242 (2013) 40–46.
[29] P. Roman, et al., Probiotics in digestive, emotional, and pain-related disorders, Behav. Pharmacol. 29 (2 and 3-Spec Issue) (2018) 103–119.
[30] E.A. Mayer, et al., Gut microbes and the brain: paradigm shift in neuroscience, J. Neurosci. : the Official Journal of the Society For Neuroscience 34 (46) (2014) 15490–15496.
[31] J.F. Cryan, et al., The microbiota-gut-brain Axis, Physiol. Rev. 99 (4) (2019) 1877–2013.
[32] C.R. Martin, et al., The brain-gut-microbiome Axis, Cellular Molecul. Gastroenterol. Hepatol. 6 (2) (2018) 133–148.
[33] B. Dalile, et al., The role of short-chain fatty acids in microbiota-gut-brain communication, Nat. Rev. Gastroenterol. Hepatol. 16 (8) (2019) 461–478.
[34] G. Morris, et al., The role of the microbial metabolites including tryptophan catabolites and short chain fatty acids in the pathophysiology of immune-inflammatory and neuroimmune disease, Mol. Neurobiol. 54 (6) (2017) 4432–4451.
[35] J.M. Yano, et al., Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis, Cell 161 (2) (2015) 264–276.
[36] J. Aresti Sanz, S. El Aidy, Microbiota and gut neuropeptides: a dual action of antimicrobial activity and neuroimmune response, Psychopharmacology 236 (5) (2019) 1597–1609.
[37] B.B. Yoo, S.K. Mazmanian, The enteric network: interactions between the immune and nervous systems of the gut, Immunity 46 (6) (2017) 910–926.
[38] D. Zheng, T. Liwinski, E. Elinav, Interaction between microbiota and immunity in health and disease, Cell Res. 30 (6) (2020) 492–506.
[39] X. Zou, et al., Gut microbes in cerebrovascular diseases: gut flora imbalance, potential impact mechanisms and promising treatment strategies, Front. Immunol. 13 (2022) 975921.
[40] Q. Yuan, et al., Lactulose improves neurological outcomes by repressing harmful bacteria and regulating inflammatory reactions in mice after stroke, Front. Cell. Infect. Microbiol. 11 (2021) 644448.
[41] M. Kang, et al., The effect of gut microbiome on tolerance to morphine mediated antinociception in mice, Sci. Rep. 7 (2017) 42658.
[42] S. Banerjee, et al., Opioid-induced gut microbial disruption and bile dysregulation leads to gut barrier compromise and sustained systemic inflammation, Mucosal Immunol. 9 (6) (2016) 1418–1428.
[43] J.P. Jacobs, J. Braun, Immune and genetic gardening of the intestinal microbiome, FEBS (Fed. Eur. Biochem. Soc.) Lett. 588 (22) (2014) 4102–4111.
[44] S.E. Power, et al., Intestinal microbiota, diet and health, Br. J. Nutr. 111 (3) (2014) 387–402.
[45] K. Lee, et al., The gut microbiota mediates reward and sensory responses associated with regimen-selective morphine dependence, Neuropsychopharmacol. Off. Publ. Am. College Neuropsychopharmacol. 43 (13) (2018) 2606–2614.
[46] C. Acharya, et al., Chronic opioid use is associated with altered gut microbiota and predicts readmissions in patients with cirrhosis, Aliment. Pharmacol. Ther. 45 (2) (2017) 319–331.
[47] J. Meng, et al., Opioids impair intestinal epithelial repair in HIV-infected humanized mice, Front. Immunol. 10 (2019) 2999.
[48] J. Meng, et al., Opioid exacerbation of gram-positive sepsis, induced by gut microbial modulation, is rescued by IL-17a neutralization, Sci. Rep. 5 (2015) 10918.
[49] M. Yu, et al., Aryl hydrocarbon receptor activation modulates intestinal epithelial barrier function by maintaining tight junction integrity, Int. J. Biol. Sci. 14 (1) (2018) 69–77.
[50] F.A. Carvalho, et al., Toll-like receptor-gut microbiota interactions: perturb at your own risk!, Annu. Rev. Physiol. 74 (2012) 177–198.
[51] M.R. Hutchinson, et al., Evidence that opioids may have toll-like receptor 4 and MD-2 effects, Brain Behav. Immun. 24 (1) (2010) 83–95.
[52] C.W. Stevens, et al., Pharmacological characterization of LPS and opioid interactions at the toll-like receptor 4, Br. J. Pharmacol. 168 (6) (2013) 1421–1429.
[53] F.A. Vicentini, et al., Intestinal microbiota shapes gut physiology and regulates enteric neurons and glia, Microbiome 9 (1) (2021) 210.
[54] B. Niesler, et al., Disorders of the enteric nervous system - a holistic view, Nat. Rev. Gastroenterol. Hepatol. 18 (6) (2021) 393–410.
[55] S. Liu, et al., Pre- and postsynaptic inhibition by nociceptin in Guinea pig small intestinal myenteric plexus in vitro, Am. J. Physiol. Gastrointest. Liver Physiol. 281 (1) (2001) G237–G246.
[56] R.A. North, Twelfth Gaddum memorial lecture. Drug receptors and the inhibition of nerve cells, Br. J. Pharmacol. 98 (1) (1989) 13–28.

[57] W. Xiao, et al., GDNF is involved in the barrier-inducing effect of enteric glial cells on intestinal epithelial cells under acute ischemia reperfusion stimulation, Mol. Neurobiol. 50 (2) (2014) 274–289.
[58] J.R. Brestoff, D. Artis, Commensal bacteria at the interface of host metabolism and the immune system, Nat. Immunol. 14 (7) (2013) 676–684.
[59] J.M. Brenchley, D.C. Douek, Microbial translocation across the GI tract, Annu. Rev. Immunol. 30 (2012) 149–173.
[60] S. Guo, et al., Secretions of Bifidobacterium infantis and Lactobacillus acidophilus protect intestinal epithelial barrier function, J. Pediatr. Gastroenterol. Nutr. 64 (3) (2017) 404–412.
[61] F. Wang, et al., Morphine induces changes in the gut microbiome and metabolome in a morphine dependence model, Sci. Rep. 8 (1) (2018) 3596.
[62] T. Trang, et al., Pain and poppies: the good, the bad, and the ugly of opioid analgesics, J. Neurosci.: Off. J. Soc. Neurosci. 35 (41) (2015) 13879–13888.
[63] J. Mao, D.D. Price, D.J. Mayer, Mechanisms of hyperalgesia and morphine tolerance: a current view of their possible interactions, Pain 62 (3) (1995) 259–274.
[64] J.M. Crow, Biomedicine: move over, morphine, Nature 535 (7611) (2016) S4–S6.
[65] P. Song, Z.Q. Zhao, The involvement of glial cells in the development of morphine tolerance, Neurosci. Res. 39 (3) (2001) 281–286.
[66] V. Jokinen, et al., Differential spinal and supraspinal activation of glia in a rat model of morphine tolerance, Neuroscience 375 (2018) 10–24.
[67] W.-T. Liu, et al., Spinal matrix metalloproteinase-9 contributes to physical dependence on morphine in mice, J. Neurosci.: Off. J. Soc. Neurosci. 30 (22) (2010) 7613–7623.
[68] D.-Q. Liu, Y.-Q. Zhou, F. Gao, Targeting cytokines for morphine tolerance: a narrative review, Curr. Neuropharmacol. 17 (4) (2019) 366–376.
[69] S. Mélik Parsadaniantz, et al., Opioid and chemokine receptor crosstalk: a promising target for pain therapy? Nat. Rev. Neurosci. 16 (2) (2015) 69–78.
[70] A. Goldstein, P. Sheehan, J. Goldstein, Unsuccessful attempts to transfer morphine tolerance and passive avoidance by brain extracts, Nature 233 (5315) (1971) 126–129.
[71] G. Corder, et al., Loss of μ opioid receptor signaling in nociceptors, but not microglia, abrogates morphine tolerance without disrupting analgesia, Nat. Med. 23 (2) (2017) 164–173.
[72] L. Liem, et al., The dorsal root ganglion as a therapeutic target for chronic pain, Reg. Anesth. Pain Med. 41 (4) (2016) 511–519.
[73] C.-m. Zhao, et al., Spinal MCP-1 contributes to the development of morphine antinociceptive tolerance in rats, Am. J. Med. Sci. 344 (6) (2012) 473–479.
[74] R.A. Mischel, W.L. Dewey, H.I. Akbarali, Tolerance to morphine-induced inhibition of TTX-R sodium channels in dorsal root ganglia neurons is modulated by gut-derived mediators, iScience 2 (2018) 193–209.
[75] S. Kawamoto, et al., The inhibitory receptor PD-1 regulates IgA selection and bacterial composition in the gut, Science (New York, N.Y.) 336 (6080) (2012) 485–489.
[76] T.K. Eisenstein, M.E. Hilburger, Opioid modulation of immune responses: effects on phagocyte and lymphoid cell populations, J. Neuroimmunol. 83 (1–2) (1998) 36–44.
[77] J. Wybran, et al., Suggestive evidence for receptors for morphine and methionine-enkephalin on normal human blood T lymphocytes, J. Immunol. 123 (3) (1979) 1068–1070.
[78] J. Ninković, S. Roy, Role of the mu-opioid receptor in opioid modulation of immune function, Amino Acids 45 (1) (2013).
[79] T.K. Eisenstein, The role of opioid receptors in immune system function, Front. Immunol. 10 (2019) 2904.
[80] L.M. Plein, H.L. Rittner, Opioids and the immune system - friend or foe, Br. J. Pharmacol. 175 (14) (2018) 2717–2725.
[81] M.L. Hanke, T. Kielian, Toll-like receptors in health and disease in the brain: mechanisms and therapeutic potential, Clin. Sci. (London, England : 1979) 121 (9) (2011) 367–387.
[82] H. Hug, M.H. Mohajeri, G. La Fata, Toll-like receptors: regulators of the immune response in the human gut, Nutrients 10 (2) (2018).
[83] M.J. Lacagnina, L.R. Watkins, P.M. Grace, Toll-like receptors and their role in persistent pain, Pharmacol. Therapeut. 184 (2018) 145–158.
[84] M. Shah, S. Choi, Toll-like receptor-dependent negative effects of opioids: a battle between analgesia and hyperalgesia, Front. Immunol. 8 (2017) 642.
[85] X. Wang, et al., Morphine activates neuroinflammation in a manner parallel to endotoxin, Proc. Natl. Acad. Sci. U.S.A. 109 (16) (2012) 6325–6330.
[86] Y. Zhang, et al., Essential role of toll-like receptor 2 in morphine-induced microglia activation in mice, Neurosci. Lett. 489 (1) (2011) 43–47.
[87] J. Meng, et al., Morphine induces bacterial translocation in mice by compromising intestinal barrier function in a TLR-dependent manner, PLoS One 8 (1) (2013) e54040.
[88] K.L. Reichelt, A.M. Knivsberg, Can the pathophysiology of autism be explained by the nature of the discovered urine peptides? Nutr. Neurosci. 6 (1) (2003) 19–28.
[89] C.P. Lázaro, M.P. Pondé, L.E.A. Rodrigues, Opioid peptides and gastrointestinal symptoms in autism spectrum disorders, Rev. Bras. Psiquiatr. 38 (3) (2016) 243–246.
[90] P. Shattock, P. Whiteley, Biochemical aspects in autism spectrum disorders: updating the opioid-excess theory and presenting new opportunities for biomedical intervention, Expert Opin. Ther. Targets 6 (2) (2002) 175–183.
[91] M. Fiorentino, et al., Blood-brain barrier and intestinal epithelial barrier alterations in autism spectrum disorders, Mol. Autism. 7 (2016) 49.
[92] T. Sakurai, et al., Degradation of food-derived opioid peptides by bifidobacteria, Benef. Microbes 9 (4) (2018) 675–682.
[93] R. Xu, et al., Altered gut microbiota and mucosal immunity in patients with schizophrenia, Brain Behav. Immun. 85 (2020) 120–127.
[94] F. Zhu, et al., Transplantation of microbiota from drug-free patients with schizophrenia causes schizophrenia-like abnormal behaviors and dysregulated kynurenine metabolism in mice, Mol. Psychiatr. 25 (11) (2020) 2905–2918.
[95] T.A. Jenkins, et al., Influence of tryptophan and serotonin on mood and cognition with a possible role of the gut-brain Axis, Nutrients 8 (1) (2016).
[96] B. Conio, et al., Opposite effects of dopamine and serotonin on resting-state networks: review and implications for psychiatric disorders, Mol. Psychiatr. 25 (1) (2020) 82–93.
[97] C.E. Gheorghe, et al., Focus on the essentials: tryptophan metabolism and the microbiome-gut-brain axis, Curr. Opin. Pharmacol. 48 (2019) 137–145.
[98] J. Weil-Fugazza, et al., Changes in total and free tryptophan levels in serum following acute morphine administration in arthritic rats, Neurochem. Int. 3 (5) (1981) 323–328.
[99] Y. Higuchi, T. Soga, I.S. Parhar, Regulatory pathways of monoamine oxidase A during social stress, Front. Neurosci. 11 (2017) 604.
[100] W. Xie, et al., Activation of brain indoleamine 2,3-dioxygenase contributes to epilepsy-associated depressive-like behavior in rats with chronic temporal lobe epilepsy, J. Neuroinflammation 11 (2014) 41.
[101] A. Stokes, et al., The contribution of obesity to prescription opioid use in the United States, Pain 160 (10) (2019) 2255–2262.
[102] H. Aslam, et al., Unravelled facets of milk derived opioid peptides: a focus on gut physiology, fractures and obesity, Int. J. Food Sci. Nutr. 71 (1) (2020) 36–49.
[103] P. Van Dyken, B. Lacoste, Impact of metabolic syndrome on neuroinflammation and the blood-brain barrier, Front. Neurosci. 12 (2018) 930.
[104] X. Zhang, et al., Minocycline can delay the development of morphine tolerance, but cannot reverse existing tolerance in the maintenance period of neuropathic pain in rats, Clin. Exp. Pharmacol. Physiol. 42 (1) (2015).
[105] L. Zhang, et al., Morphine tolerance is attenuated in germfree mice and reversed by probiotics, implicating the role of gut microbiome, Proc. Natl. Acad. Sci. U.S.A. 116 (27) (2019) 13523–13532.
[106] Z. Chen, et al., The ameliorative effects of isorhynchophylline on morphine dependence are mediated through the microbiota-gut-brain Axis, Front. Pharmacol. 12 (2021) 526923.
[107] Z. Chen, et al., Ginsenoside Rg1 mitigates morphine dependence via regulation of gut microbiota, tryptophan metabolism, and serotonergic system function, Biomed. Pharmacother. = Biomedecine & Pharmacotherapie 150 (2022) 112935.