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The "culture" of pain control: A review of opioid-induced dysbiosis (OID) in antinociceptive tolerance

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Abstract

It is increasingly recognized that chronic opioid use leads to maladaptive changes in the composition and localization of gut bacteria. Recently, this "opioid-induced dysbiosis" (OID) has been linked to antinociceptive tolerance development in preclinical models and may therefore identify promising targets for new opioid-sparing strategies. Such developments are critical to curb dose escalations in the clinical setting and combat the ongoing opioid epidemic. In this article, we review the existing literature that pertains to OID, including the current evidence regarding its qualitative nature, influence on antinociceptive tolerance, and future prospects.

Keywords

analgesia; antibiotics; antinociception; dorsal root ganglia; dysbiosis; microbiome; morphine; opioid; tolerance

INTRODUCTION

In October 2017, the United States government declared a state of public health emergency in response to the growing prescription opioid epidemic. Social, political, and medical rhetoric has recently demanded improvements in clinical pain control, prompting the publication of the "Guideline for Prescribing Opioids for Chronic Pain" by the Centers for Disease Control and Prevention (CDC) in March, 2016.[31] Over the last decade, opioid use in the US has risen dramatically, with the number of prescriptions written by health care providers in 2012 (~259 million) exceeding the total adult population (~240 million). Opioid-related emergency department visits and mortality rates have concurrently increased, with over 165,000 deaths by overdose during 1999–2014. Despite this hardship, opioid analgesics remain an essential component of modern healthcare as the gold standard of therapy for moderate to severe pain. Since complete elimination of opioids would be impractical and even detrimental, there has been a great push to identify new opioid-sparing

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strategies, such as reducing analgesic tolerance development[4,27]. With mounting evidence suggesting that opioids alter the composition and localization of gut microbes, questions have been raised of whether opioid-induced dysbiosis (OID) influences analgesic tolerance development. Indeed, the physiological influence of gut microbes is both robust and widespread – prominent examples include gastrointestinal disorders such as irritable bowel syndrome (IBS)[70] and inflammatory bowel diseases (IBD)[64,116], metabolic and endocrine states such as obesity[122] and type I/II diabetes mellitus[2,88], and even maladies of central origin such as anxiety and depression[71,106].

OPIOID-INDUCED DYSBIOSIS

Composition

Qualitative analyses of opioid-induced dysbiosis (OID) are still developing, but numerous independent investigators have found that rodents with chronic morphine exposure display significant shifts in the composition of commensal bacteria (Table 1). Meng *et al.* (2015) found a significant enhancement in the abundance of Gram-positive *Staphylococcus* and *Enterococcus* in mice[75], while an order analysis by Kang *et al.* (2017) further indicated a reduction of *Bacteroidetes* (*Bacteroidales*) and *Firmicutes* (*Clostridiales* and *Lactobacilliales*) with enrichment of *Proteobacteria* (*Enterobacteriales*).[56] Many actions of morphine likely promote these shifts, including inhibition of gut mucus and bicarbonate secretion[95], prolongation of intestinal transit time[8,110], inhibition of bile acid secretion[118], and suppression of immune surveillance[17]. In addition to compositional changes, morphine has also been noted to modify the characteristics of individual genera or species. For example, a focused study by Wang *et al.* (2018) observed enrichment of specific pathogenic genera of *Flavobacterium*, *Enterococcus*, *Fusobacterium*, *Sutterella*, and *Clostridium*[129], while Banerjee *et al.* (2016) noted a net expansion in the diversity of *Firmicutes* (*Enterococcaceae*, *Staphylococcaceae*, *Bacillaceae*, *Streptococcaceae*, and *Erysipelotrichaceae*) and a reduction of bile-deconjugating bacterial strains[9]. An additional study by Babrowski *et al.* (2012) indicated that chronic morphine activates virulence in *Pseudomonas aeruginosa*.[7] These findings present a sound initial framework, but further studies are certainly warranted to enhance the resolution of these findings and assess whether they are consistent between different opioids. Particular attention should be paid to the treatment protocol in future studies as Lee *et al.* (2018) observed that escalating intermittent morphine increased abundance of *Ruminococcus* and decreased *Lactobacillus* in mouse fecal matter, while continuous exposure instead increased *Clostridium* and *Rikenellaceae*.[63] Nonetheless, the consensus among existing findings is encouraging and provides some insight about how the biochemical milieu of the gut might be influenced by chronic opioid exposure.

Gram-negative *Bacteroides* are the most abundant microbes in the distal ileum and colon. They are generally considered to be gut-protective, in part due to modulation of immune reponses.[102] They are broadly proficient digesters of carbohydrates and fiber, yielding short-chain fatty acids (SCFAs) that quell inflammation[109] and promote impermeability of the epithelial barrier[87]. However, individual *Bacteroides* species demonstrate additional mechanisms of immunomodulation. For example, polysaccharide A (PSA) from *Bacteroides*

fragilis has been observed to enhance the abundance of helper T cells and reduce inflammation[73,74]. This resonates with findings that the relative abundance of *B. fragilis* is reduced in patients with IBD[50,80]. *Bacteroides thetaiotaomicron* also displays gut-protective properties, having been noted to alter mucosal gene expression in a manner promoting barrier impermeability, nutrient absorption, cellular maturation, and angiogenesis.[47,93] Collectively, these findings are consistent with studies of OID in which chronic morphine induced a relative reduction of *Bacteroidetes* that correlated with an increase in pro-inflammatory cytokine release and breakdown of the epithelial barrier.[56]

Gram-positive *Firmicutes* (*Clostridiales* and *Lactobacilliales*) are also of reduced abundance in mice with chronic morphine exposure. *Clostridia*, although better known for their pathogenic species (e.g., *Clostridium difficile*, *tetani*, *botulinum*, and *perfringens*), are also highly abundant in the healthy distal ileum and colon.[102] Commensal *Clostridia* demonstrate a significant level of anti-inflammatory activity and have significantly reduced abundance in patients with IBD.[102] This anti-inflammatory action has primarily been attributed to fiber metabolism, SCFA production (especially butyrate), and induction of regulatory T cells that produce TGFβ and IL-10.[6,39] *Lactobacillus* species are typically less abundant than both *Bacteroides* and *Clostridia*, but are among the most thoroughly tested and commonly recommended probiotic agents. Their anti-inflammatory action and therapeutic potential in IBD have been extensively documented and reviewed.[84] These findings suggest that the reduced abundance of *Firmicutes* with chronic morphine may also contribute to pro-inflammatory action in the gut.

Gram-positive *Proteobacteria* (*Enterobacteriales*) demonstrate enhanced abundance in mice with chronic morphine exposure. These microbes are notably enriched in a number of disease states (both intra- and extra-intestinal), and have been suggested as biological indicators of dysbiosis.[107] Inflammation appears to be a fundamental component of *Proteobacteria*-related diseases[94], and an enhanced relative abundance is observed in patients with IBD[38,43,101]. A recent study indicated that intestinal biopsy samples possess a greater abundance of *Proteobacteria* than fecal samples[41,80], suggesting that their expansion favors regions with close mucosal proximity. This enhances the potential for functional impacts on mucosal tissues, and may be an important component of the pro-inflammatory activity occurring with chronic morphine exposure.

Genomic and proteomic analyses with greater depth and coverage (e.g., bacteria, fungi, viruses, etc.) are undoubtedly necessary to capture the full breadth of OID. Nonetheless, the current evidence in mice suggests a shift in GI microflora toward a phenotype that is pro-inflammatory and deleterious to epithelial barrier integrity. It remains unclear whether these findings will translate to humans. In terms of composition, the gut microbiomes of mice and humans are broadly similar with *Bacteroidetes* and *Firmicutes* (*Clostridiales* and *Lactobacilliales*) dominating over numerous other minor phyla.[66,123,134] These minor phyla, however, have demonstrated differences in composition and interactions with host tissues.[81] For example, Gram-positive segmented filamentous bacteria (SFB) with pro-inflammatory properties are enriched in the terminal ileum of rodents[51,59], and 85% of the other minor phyla in mice are completely absent in humans[65]. Whether the minor differences will transcend the overarching similarities remains to be determined, and studies to date have not

been designed to address this question. Dysbiosis has been noted in patients with substance use disorders (heroin among them)[135], but no study has examined for a main effect of opioid use. Such a design, however valuable, may be complicated by the frequency of polysubstance use in opioid abusers. A better controlled design may involve patients with clinical opioid use. It has been speculated that opioids are linked to enhanced microbial virulence in critically ill ICU patients, but further investigations are needed[22] and may be limited by the duration of use. Chronic outpatient users may provide an ideal study group (see Experimental Paradigms, C*olon Tissue Supernatants*).

Translocation

Recent findings have also linked opioid exposure to the translocation of gut microbes. Chronic morphine has been noted to produce a "leaky" gut in mice by compromising the integrity of epithelial tight junctions through mechanisms involving TLR-2/4 activation, IL-17A release (driven by IL-1β and IL-23), and redistribution of tight junction protein zonula occludens-1 (ZO-1).[56,75] The resulting increase in epithelial permeability allows translocation of luminal bacteria to the gut wall, mesenteric lymph nodes (MLN), blood, liver, and spleen. The intestinal histology in these mice reveals neutrophilic infiltration (i.e., inflammation) and structural disruption of the mucosal crypts and lamina propria. Further investigation is certainly necessary, but similar tissue destruction has been noted in the intestines of human opioid abusers (Meng et al., 2015, Figure 6J).[75] Future investigations might also consider the antimicrobial peptides and local immune responses in Peyer's patches that are well known to contribute to the physical barrier of the gut.

Immunomodulation

Regulation of immune responses by opioids is exceptionally complex, stimulating some pathways, inhibiting others, and often demonstrating dual effects. The immunosuppressive action of opioids on T-cells, B-cells, and macrophages is well documented[82,98,125], yielding reductions in peripheral inflammatory mechanisms such as neutrophil chemotaxis and extravasation[68,78], macrophage recruitment, and TNF-α release from mast cells[98]. This is a widely accepted postulate, although it has been noted that many characterization studies are performed *in vitro* or utilize an acute morphine paradigm that may limit their translation to chronic models *in vivo*.[68] Perhaps paradoxically, mice with chronic morphine exposure actually demonstrate increased activation of innate immune responses in the gut, including neutrophil infiltration and expression of IL-1β.[56,76] This suggests that any immunosuppressive effects of morphine in the gut are overshadowed by local pro-inflammatory stimuli. This concurs with findings by Zhang *et al.* (2019) that chronic morphine exposure in mice enhances expression of IL-6, IL-1β, and TNF-α.[138] Pro-inflammatory cytokine production (TNF-α, IL-1β, IL-6, and IL-8) has also been noted in patients on methadone maintenance therapy, with TNF-α and IL-6 concentrations that correlate with methadone usage.[21]

A potentially important source of this cytokine release is glial cells (including microglia and astrocytes). Glial cell activation by morphine has been described in the dorsal horn of the spinal cord[49,53,131], in enteric glia[14], and in satellite glia of the dorsal root ganglia[13,52]. Glial activation mechanisms are thought to contribute to the pathogenesis of analgesic

tolerance[25,32,44], and are discussed further in the corresponding section below (see Analgesic tolerance).

Experimental paradigms

Axenic animals—Many recent studies of the microbiome have used animals reared in germ-free environments as a model of bacterial clearance. These paradigms should be implemented cautiously as numerous developmental anomalies have been documented in these subjects that may confound the outcomes. The "natural" relationship with bacteria begins during birth, is pervasive throughout life, and serves numerous key physiological roles. These include 1) production of short-chain fatty acids (SCFA), vitamins, essential amino acids, and neurotransmitters, 2) modulation of gut epithelial gene expression, and 3) transformation of bile acids. Animals raised in germ-free environments exhibit profound developmental immaturity of virtually every body system[108], and a short list of the dietary and metabolic impacts include the requirement of exogenous vitamin K, decreased body fat, increased cholesterol, decreased total blood volume, and decreased basal metabolic rate. Immune insufficiency is particularly prominent in these animals, with some of the greatest immune deficits being noted in the gut (e.g., decreased immune tissue density, small and sparse Peyer's patches, and small mesenteric lymph nodes that lack germinal centers). These findings offer high face validity given that the microbiome is in intimate contact with gut tissues in the native state. Indeed, gastrointestinal immaturity is a general theme in axenic animals, with eminent examples found in the small intestine (reduced osmolarity, elevated oxygen tension and electripotential, decreased total mass and surface area for absorption, narrowing of villi, and truncation of ileal villi) and cecum (increased volume, reduced wall thickness, decreased spontaneous contraction, and increased alkalinity). While germ-free mice may provide some utility in studies of OID, investigators should be advised to interpret results cautiously and correct for confounding variables as able.

Antibiotics—Bacterial clearance can alternatively be achieved through the use of antibiotics. This allows subjects to achieve developmental adulthood prior to testing, and circumvents the issues noted in axenic animals. It allows targeted elimination of bacteria based on the coverage spectrum of the antibiotic(s) and creates potential for within-subjects comparisons of responses before and after bacterial clearance. However, the potential for drug-drug and other metabolic interactions should always be considered when using antibiotics, and investigators should attempt to minimize these factors when able. In this regard, antibiotics with low oral bioavailability offer some advantage, but it remains important to consider how opioid-induced breakdown of the gut epithelial barrier may influence drug uptake. Selecting antibiotics with negligible production of metabolites and off-target binding can minimize the impact of inadvertent absorption.

An example that meets the aforementioned criteria is vancomycin. Vancomycin has virtually 0% oral bioavailability and is eliminated unchanged in the urine even if small amounts enter the bloodstream. Importantly, it does not demonstrate significant conversion to metabolites *in vivo*.[72] It bactericidal action is restricted to Gram-positive organisms by its mechanism of action, binding the D-Ala-D-Ala component of the cell wall precursor peptidoglycan. Mammalian cells do not possess this D-Ala-D-Ala component or have cell walls, and

evidence is lacking for significant off-target binding.[99] Though some impacts on gut physiology have been noted with vancomycin administration (e.g., modulation of permeability[121]), much of this can be attributed to reductions in Gram-positive bacteria.

Fecal matter transplant (FMT)—The introduction of foreign fecal matter into the gut has emerged as a robust method for modifying floral composition *in vivo*. This method is well known for its efficacy in treating pseudomembranous colitis caused by *Clostridium difficile* infection[57,58], but significant benefits have also been noted for IBD, IBS, and constipation.[3,5,15,16,89] A recent investigation by Banerjee *et al.* (2016) demonstrated that the microbial dysbiosis and gut barrier disruption associated with chronic morphine exposure could be prevented by FMT from placebo-treated counterparts.[9] More recently, Zhang et al. (2019) noted that fecal matter from mice with chronic morphine exposure induces morphine tolerance in germ-free mice.[138]

Probiotics—A less robust but easily translated model of microbial restructuring is probiotic treatment. Numerous bacterial genera have demonstrated efficacy in this regard, but *Lactobacillus* and *Bifidobacteria* are among the most commonly utilized. Their anti-inflammatory action and therapeutic potential in IBD have been extensively documented[84], and recent evidence suggests that they may be able to reduce visceral pain associated with conditions like IBS[96,117]. Probiotic supplementation (e.g., *Lactobacillus rhamnosus* GG) may therefore be a viable method of rescuing the deleterious shifts in microbial composition that occur with chronic morphine exposure. Recent investigations demonstrate that probiotic treatment is sufficient to reverse tolerance development[138], and have the major advantage of being readily translated to a human patient population.

Colon tissue supernatants—The use of supernatants from colon tissue samples has arisen as an innovative and powerful paradigm for investigating the mechanisms of peripheral nociception in both mice and human patients.[1,10,20,77,124] Full-circumference colon tissue segments are resected and incubated in culture medium such that biochemical mediators in the gut wall (e.g., bacterial products and pro-inflammatory cytokines) leach out into the culture medium. The liquid supernatant may then be transferred to a target cell population (e.g., naïve DRG cultures) to assess the impact of gut-localized mediators on neuronal excitability. A recent study observed that colon tissue supernatants from mice with chronic morphine exposure induced tolerance and hyperexcitability in naïve mouse DRG neurons.[77] Oral vancomycin treatment in this paradigm was sufficient to block tolerance development, but not hyperexcitability. This evidence suggests that tolerance development *in vivo* involves a peripheral, gut-mediated component in addition to the central mechanisms that have been extensively described.

A major cited advantage of using colon tissue supernatants is the ease with which translational relevance can be established. To this end, Valdez-Morales *et al.* (2013) demonstrated that supernatants (and thus gut-derived mediators) from human mucosal biopsy samples could be applied to mouse DRG neurons to examine the influence on neuronal excitability. In the absence of human DRG donors, this offers a practical method to establish translational relevance prior to the rigorous and costly process of identifying the underlying biochemical mechanisms involved. In a similar fashion, naïve mouse DRG

neurons may be exposed to supernatants from human colon biopsy samples in patients with a history of chronic opioid use to examine the influence on tolerance development. Patients with constipation-predominant irritable bowel syndrome (IBS-C) represent an ideal population for such an investigation for numerous reasons: 1) the prevalence of opioid use for clinical pain is likely to be significant, 2) colonoscopy with biopsy is commonly performed in these patients for pathological assessments and the samples often lack confounding pathology (e.g., malignancy or IBD), 3) evidence indicates that control IBS-C colon supernatants will not impact excitability in DRG neurons (Valdez-Morales *et al.*, 2013), and 4) the incidence of IBS-C diagnoses at most major medical centers is likely to be high. Such an experiment should include standardization and/or stratification of patients to the extent possible, and sources of variation should be considered and compensated for. This may include, but is not limited to, the opioid regimen (specific drug, dosage, presence of additives, rate of release, and duration of use), past medical history (e.g., recent antibiotic administration), and demographics (e.g., age, sex, and race). With regard to variability among opioids, it is notable that many produce cross-tolerance with morphine and modulation of tolerance may be observed even if stratification is not possible.

ANALGESIC TOLERANCE

Behavioral tolerance

With mounting evidence supporting the notion of OID, questions have been raised of how the liberated bacterial products and secondary pro-inflammatory responses (particularly in the gut wall) may influence the adaptive cellular processes associated with antinociceptive tolerance development. Indeed, bacterial N-formylated peptides have been noted to directly activate sensory neurons[24] and inflammation is well established to sensitize primary afferent pain fibers and modulate nociceptor signaling/gene expression in various paradigms.[12,33,42,67,114,128] Inflammation has also been shown to result in significant plasticity of opioid signaling, including receptor expression, G protein signaling, and receptor trafficking.[139] In fact, a recent study found that TNBS-induced colitis hastens the development of antinociceptive tolerance in mice with chronic morphine exposure.[61] This certainly resonates with the qualitative evidence of OID, and is supported by years of clinical evidence demonstrating a synergistic relationship in the analgesic properties of nonsteroidal anti-inflammatory drugs (NSAIDs) and opioids.[60,137]

Investigators have now begun to assess the role of OID in antinociceptive tolerance by utilizing the variety of experimental paradigms described above (see Experimental paradigms). Kang *et al.* (2017) noted that selective elimination of Gram-positive organisms via oral vancomycin attenuated morphine tolerance development *in vivo*.[56] This effect was only modestly enhanced using broad-spectrum antibiotics with coverage for Gram-negative bacteria; so while both may have some impact on tolerance development, Gram-positive organisms appear to be the primary offenders. This resonates with evidence that chronic morphine exposure in mice selectively promotes Gram-positive sepsis.[75] A study by Zhang *et al.* (2019) also noted attenuation of tolerance using a non-absorbable broad-spectrum antibiotic cocktail and a tolerance paradigm of escalating-dose morphine injections. This study further noted attenuation of tolerance in germ-free mice, induction of tolerance via

FMT from mice with chronic morphine exposure, and reversal of tolerance via probiotic treatment.[138]

The implication of Gram-positive microbes certainly warrants further investigation. Gram-positive and Gram-negative bacteria are, by definition, differentiated based on the retention of crystal violet dye, which depends on the physical and chemical composition of the cell wall. Lipoteichoic acid (LTA) is a major constituent of the Gram-positive cell wall that is known to bind TLR-2, inducing NF-κB expression and downstream pro-inflammatory activity. The Gram-negative correlate of LTA is lipopolysaccharide (LPS, endotoxin).[112,126] LPS is present in the outer membrane of the cell wall and binds TLR-4 to induce NF-κB signaling. Despite their similarities, nuances have been noted in the responses elicited by LTA and LPS, including cytokine induction profiles, leukocyte epithelial adhesion, and reorganization of tight junction proteins.[19,37,54] TLR-2 is the only Toll-like receptor that has been observed to modify the arrangement of tight junction proteins in the gut epithelial barrier,[18] and may therefore contribute to the compromise of epithelial barrier integrity that is noted with chronic morphine. Indeed, this finding was mitigated in studies using vancomycin. Still, the interactive effect with Gram-negative microbes is apparent, and may involve LPS-induced cytokine release from enteric and/or DRG satellite glia.[14]

Cellular tolerance and signaling

A critical future direction is to identify the cell populations and signaling mechanisms that underlie the behavioral findings. Primary afferent neurons are the first-order components of nociceptive signaling and an immediately accessible component of the pain pathway for translocating bacteria. These cells have soma localized to the DRG and terminal processes that innervate peripheral regions including the gut wall. The importance of DRG nociceptors in antinociceptive tolerance was recently demonstrated by Corder *et al.* (2017) utilizing a mouse model with TRPV1 promoter-driven Cre recombinase and a loxP-flanked OPRM1 gene that conditionally deleted μ-OR expression in neurons of TRPV1 lineage (i.e., nociceptors).[28] When exposed to morphine, these mice exhibited reductions in tolerance and opioid-induced hypernociception (OIH) development, suggesting that these effects are driven by μ-OR activation on DRG nociceptors. Peripheral mechanisms play a key role in nociceptive sensation, and opioid receptors on DRG neurons have been suggested to account for 50–100% of the antinociceptive effect of systemic opioids.[111] Indeed, even peripherally restricted opioids like loperamide demonstrate significant antinociceptive properties along with tolerance and OIH development.[46,55,127] With regard to OID, Kang *et al.* (2017) noted that oral vancomycin prevented morphine tolerance in DRG neurons isolated from lumbosacral spinal levels. This theme persisted in a further study examining the inactivation kinetics of tetrodotoxin-resistant (TTX-R) Na^+ channels ($Na_V1.8/1.9$).[77] Of note, these observations in peripheral afferents do not dispute central mechanisms of opioid tolerance, but rather imply that additional mechanisms are contributing.

An appealing target for further investigation of TTX-R Na^+ channels is β-arrestin2 (β-Arr2). μ-ORs are classical $G_{i/o}$-coupled receptors that dissociate G_α and $G_{\beta\gamma}$ subunits when activated. The G_α subunit inhibits the adenylyl cyclase (AC)/cAMP/PKA pathway. The $G_{\beta\gamma}$ component has a number of established effects, including 1) activation of GIRK

channels[100,119,120], 2) inhibition of voltage-gated Ca^{2+} channels[79,104], 3) recruitment of GRK2/3, 4) activation of the MAPK cascade (Ras, Raf, MEK, ERK1/2, JNK, p38) in a PI3K-/c-Src-dependent manner[90,91,133], 5) activation of the PLC/PKC pathway, and 6) stimulation of CaMKII[92]. GRK2/3 recruitment to the μ-OR results in phosphorylation of the agonist-bound receptor, inducing a μ-OR conformation with greater affinity for β-Arr2. β-arrestins are ubiquitously expressed proteins that function to desensitize (or "arrest") the vast majority of G protein-coupled receptors.[30] Binding of β-Arr2 creates steric hindrance that prevents μ-OR coupling with G proteins and targets the μ-OR to clathrin-coated pits for internalization. These canonical actions play key roles in morphine desensitization and tolerance development. Recruitment of β-Arr2 to the μ-OR modulates channel activity by diverting β-Arr2 away from other molecular targets. This effect has been noted to enhance the sensitivity of TRPV1 channels.[97] Whether this mechanism also impacts TTX-R Na^{+} currents, directly or indirectly, remains to be evaluated. Recent evidence suggests that β-Arr2 also serves as a scaffolding protein for activation of various intracellular kinase cascades, including MAPK.[30,34] MAPK can phosphorylate multiple cytoplasmic and nuclear targets (e.g., CREB) to modulate transcriptional events. It is therefore probable that this β-Arr2/MAPK pathway is involved in the cellular adaptive processes that take place with chronic opioid exposure to modulate excitability and tolerance. Finally, β-Arr2 appears to play a role in inflammation and immunity.[11,35,45] In this regard, it has been noted to inhibit the NF-κB pathway by preventing the phosphorylation and degradation of IκBα[40,113], interfering with LPS-induced NF-κB activation, and reducing TRAF6 autoubiquitination.[130] This may have important implications for cytokine production pathways that involve nuclear translocation of NF-κB (e.g., TLR/MyD88), and may play a role in morphine tolerance development.[56,75,76]

Glial activation

The influence of gut dysbiosis on tolerance in primary afferent neurons raises questions regarding the involvement of glia. Opioid-induced glial activation has been described in the dorsal horn of the spinal cord[49,53,131], enteric glia[14], and satellite glia of dorsal root ganglia.[13,52] Glial activation releases numerous cytokines, chemokines, and neurotropic factors into the surrounding environment[26,36,62,85] and is thought to contribute to analgesic tolerance development[25,32,44]. Indeed, studies using non-selective glial inhibitors (e.g., minocycline[29], fluorocitrate[132], and propentofylline[115]) have noted attenuation of tolerance *in vivo*.[48] Bacterial products like LTA and LPS are well established to induce glial activation and inflammation.[14,69,83,136] Given that OID involves bacterial translocation, the release of bacterial products may contribute to tolerance development via glial activation. Satellite and spinal glia have direct contacts with somatic afferents in pain pathways, and enteric glia could sensitize somatic afferents indirectly via visceral afferents and satellite glia. Future investigations should note that opioid receptor activation *per se* has been linked to expression of pro-inflammatory mediators.[26,85,140] This mechanism should be regarded as a potential confounder.

CONCLUDING REMARKS

The role of opioid-induced dysbiosis (OID) in modulation of pain control is increasingly evident. Further characterization studies are necessary, particularly to improve the resolution of compositional analyses (e.g., advanced genomics and proteomics). This will greatly facilitate the identification of causal mechanisms in tolerance development. Nonetheless, the consensus of present literature points to a few prevailing themes regarding OID: 1) a shift in gastrointestinal microflora toward a phenotype that is pro-inflammatory and deleterious to epithelial barrier integrity, 2) translocation of microbes to the gut wall and other extra-intestinal sites, and 3) a pervasive correlation between dysbiosis, activation of pro-inflammatory cascades, and development of antinociceptive tolerance. Current findings further suggest that peripheral components of the pain pathway (i.e., primary afferent neurons) significantly contribute to this process (Figure 1). This presents with great face validity given the close proximity of the microbiome with peripheral tissues; however, it does not dispute the potential co-involvement of central mechanisms of tolerance, and further evaluation of this topic is necessary.

Beyond antinociceptive tolerance, it is also worth noting that the mechanisms of opioid-induced hypernociception (OIH) are also thought to contribute to dose escalations in the clinical setting.[4,27] Even in the absence of *bona fide* hyperalgesia, the mechanisms involved in OIH may be activated, reducing the analgesic potency of opioids.[23,25,103] “Tolerance” is often used as a catch-all term in instances where a diminished response is noted, but the underlying mechanisms are unique and should be approached accordingly. At present, data are insufficient to determine whether OID impacts the mechanisms of OIH, but studies using vancomycin to eliminate Gram-positive organisms have not observed any influence in the setting of chronic morphine exposure.[77]

Regarding the opioid epidemic, the need for novel opioid-sparing strategies is self-evident. New therapeutic methods that reduce the dose and duration of opioid use are essential, as each is predictive of physical dependence, addiction, and overdose.[86,105] Elucidating the mechanisms of OID in pain control, while complex, will advance our understanding of opioid pharmacology and take strides toward improving patient outcomes.

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Abbreviations

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
β-Arr2	β-arrestin2

Ca^{2+}	divalent calcium
CaMKII	Ca^{2+}/calmodulin-dependent protein kinase II
cAMP	cyclic adenosine monophosphate
CNS	central nervous system
CREB	cAMP response element-binding protein
DRG	dorsal root ganglion
ERK	extracellular signal-regulated kinase
FACS	fluorescence-activated cell sorting
GIRK	G protein-coupled inwardly-rectifying potassium
GRK	G protein-coupled receptor kinase
JNK	c-Jun N-terminal kinase
K^+	monovalent potassium
MAPK	mitogen-activated protein kinase
MEK	mitogen-activated protein kinase kinase
mRNA	messenger ribonucleic acid
Na^+	monovalent sodium
NMDA	N-Methyl-D-aspartate
OIH	opioid-induced hypernociception
PI3K	phosphoinositide 3-kinase
PKA	protein kinase A
PKC	protein kinase C
PLC	phospholipase C
s.c.	subcutaneous
TEA	tetraethylammonium
TRPV1	transient receptor potential cation channel subfamily V member 1
TTX-R	tetrodotoxin-resistant
TTX-S	tetrodotoxin-sensitive
V_t	action potential threshold

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Highlights

- Chronic opioid exposure results in opioid-induced dysbiosis (OID) of gut bacteria
- OID is characterized by pro-inflammatory gut bacteria and leaky epithelial barriers
- OID correlates with antinociceptive tolerance development
- Peripheral mechanisms contribute to OID-related tolerance development

Perspective

This article reviews the current literature on opioid-induced dysbiosis (OID) of gut bacteria, including its qualitative nature, influence on antinociceptive tolerance, and future prospects. This work may help identify targets for new opioid-sparing strategies.

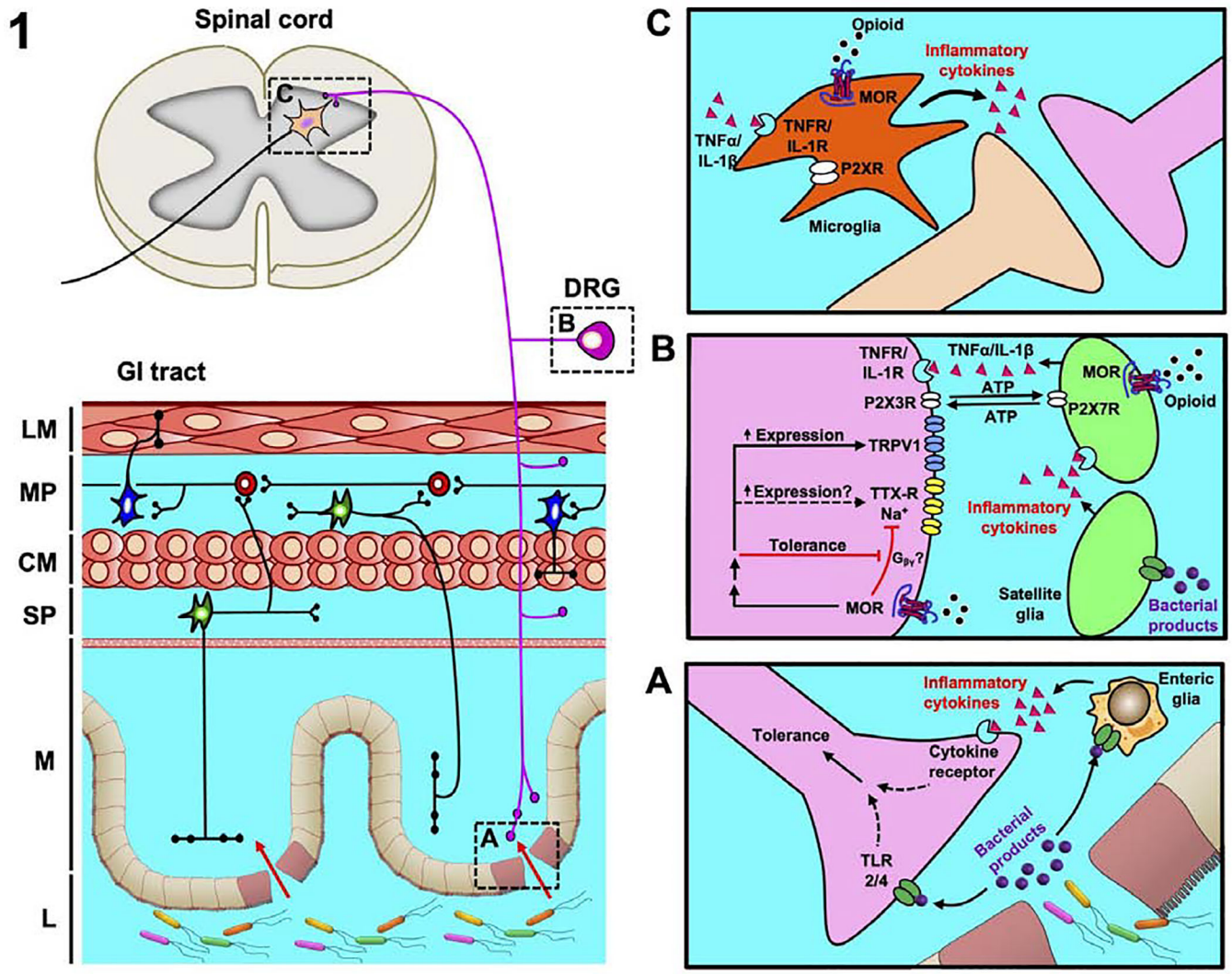

Figure 1: Opioid-induced dysbiosis (OID) modulates tolerance development in primary afferent neurons.

A representative schematic depicts the interactions between dysbiotic gut microbes and gut-innervating primary afferent neurons. This exhibit captures the mechanisms discussed in this article, and is not intended to be comprehensive. Layers of the gastrointestinal (GI) tract have been labeled for clarity – (L) lumen, (M) mucosa, (SP) submucosal plexus, (CM) circular muscle layer, (MP) myenteric plexus, (LM) longitudinal muscle layer. Chronic opioid exposure alters the composition of gut microbes and compromises epithelial barrier integrity, promoting bacterial translocation to the gut wall and other extra-intestinal sites. Studies of OID demonstrate a pervasive correlation between the subsequent release of biochemical mediators (e.g., bacterial products, pro-inflammatory cytokines) and the development of antinociceptive tolerance. The dorsal root ganglion (DRG) nerve terminals **(A)**, cell soma **(B)**, and dorsal horn synapses **(C)** are all sites that have been implicated in this process and/or demonstrate promising prospects for further investigation.

Table 1:

Preclinical studies in mice indicate that chronic morphine exposure alters the composition of gut microbiota.

Treatment protocols indicate the route (s.c. pellet implantation vs. i.p. injection), dosage, frequency, and duration of morphine exposure.

Finding	Treatment protocol	Sample	Strain	Reference
↑ Pathogenic genera (Flavobacterium, Enterococcus, Fusobacterium, Sutterella, Clostridium)	25 mg pellet (3 days)	Fecal DNA	C57BL/6J	Wang et al., 2018
↑ Ruminococcus ↓ Lactobacillus	10 mg/kg i.p. (every 12 hours, 4 days)	Fecal DNA	C57BL/6J	Lee et al., 2018
↑ Clostridium ↑ Rikenellaceae	25 mg pellet (4 days)			
↑ Proteobacteria (Enterobacteriales) ↓ Bacteroidetes (Bacteroidales) ↓ Firmicutes (Clostridiales, Lactobacilliales)	75 mg pellet (5 days)	Fecal DNA	Swiss Webster	Kang et al., 2017
↑ Diversity of Firmicutes (Enterococcaceae, Staphylococcaceae, Bacillaceae, Streptococcaceae, Erysipelotrichaceae) ↓ Bile-deconjugating bacterial strains	25 mg pellet (5–6 days)	Fecal DNA	C57BL/6J	Banerjee et al., 2016
↑ Staphylococcus ↑ Enterococcus	25 mg pellet (≤4 days)	Fecal DNA	C57BL/6	Meng et al., 2015
↑ Virulence of Pseudomonas aeruginosa	25 mg pellet (36 hours)	Cecal tissue DNA	C57BL/6	Babrowski et al., 2012