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NEUROPSYCHOPHARMACOLOGY REVIEWS

Neuronal and glial factors contributing to sex differences in opioid modulation of pain

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Morphine remains one of the most widely prescribed opioids for alleviation of persistent and/or severe pain; however, multiple preclinical and clinical studies report that morphine is less efficacious in females compared to males. Morphine primarily binds to the mu opioid receptor, a prototypical G-protein coupled receptor densely localized in the midbrain periaqueductal gray. Anatomical and physiological studies conducted in the 1960s identified the periaqueductal gray, and its descending projections to the rostral ventromedial medulla and spinal cord, as an essential descending inhibitory circuit mediating opioid-based analgesia. Remarkably, the majority of studies published over the following 30 years were conducted in males with the implicit assumption that the anatomical and physiological characteristics of this descending inhibitory circuit were comparable in females; not surprisingly, this is not the case. Several factors have since been identified as contributing to the dimorphic effects of opioids, including sex differences in the neuroanatomical and neurophysiological characteristics of the descending inhibitory circuit and its modulation by gonadal steroids. Recent data also implicate sex differences in opioid metabolism and neuroimmune signaling as additional contributing factors. Here we cohesively present these lines of evidence demonstrating a neural basis for sex differences in opioid modulation of pain, with a focus on the PAG as a sexually dimorphic core of descending opioid-induced inhibition and argue for the development of sex-specific pain therapeutics.

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INTRODUCTION

Pain is a submodality of somatosensation that is necessary for survival, yet pain may also occur and/or persist in the absence of actual or threatened tissue damage. Chronic pain, defined as pain lasting more than 3–6 months, will affect more than one in three Americans in our lifetime [1]. In the most recent National Health Interview Survey, it was reported that 55.7% of adults in the United States experienced acute pain within the past 3 months, while 11.2% reported experiencing chronic pain (reported as *daily* pain for 3 months) [2]. The prevalence and ineffective management of chronic pain is burdensome not only to the individual sufferer, but also to family members, employers, and the healthcare system [3]. Chronic pain disproportionately affects women who are 2–3× more likely to be diagnosed with and treated for chronic pain disorders, including fibromyalgia, headaches and migraine, temporomandibular joint disorder pain, irritable bowel syndrome, and osteoarthritis [4–9].

Despite their introduction over five millennia ago, opioids remain the most common therapeutic treatment for the management of chronic pain [10, 11]. It has been reported that as many as 3–5% of adults in the United States are prescribed long-term opioid therapy [12]. A recent survey of persistent pain patients showed that 60% were prescribed morphine, 37% fentanyl, and 2.5% buprenorphine, with an average length of treatment of 105 days [13]. While opioids are highly effective and often *necessary* for the management of many persistent and severe pain conditions, prolonged use results in decreased analgesic efficacy (i.e., tolerance) and the requirement for steadily larger doses of opioids for pain management (i.e., dose-escalation) [14, 15]. Opioids also elicit a multitude of undesired central nervous system-mediated side effects including sedation, decreased motor coordination and cognition, depressed pulmonary ventilation, reduced cardiac output, increased risk of mortality by overdose [16, 17], and severe constipation requiring secondary pharmaceutical interventions [18]. For some, pain management with opioids results in opioid-induced *hyperalgesia*, a paradoxical effect of opioids resulting in enhanced sensitivity to pain [19].

From these reports, it is discernible that the development of tolerance and the requirement for dose-escalation not only leads to many or all of the aforementioned negative side effects but also heightens the risk of developing opioid addiction in chronic pain patients. This issue has been at the forefront of recent headlines as reporting heightens amidst the current opioid crisis in America. According to the Department of Defense Survey of Health-related Behaviors in active military service members, a demographic currently dealing with an increase in chronic pain patients, there was a *tripling* of prescription drug abuse between 2005 and 2008 [20]. Curbing addiction to prescription painkillers is now a top priority of the Office of The Army Surgeon General (May 2010) [177].

The analgesic properties of systemically administered opioids are mediated primarily via the endogenous descending pain modulatory circuit, consisting of the midbrain periaqueductal gray (PAG) and its descending projections to the rostral ventromedial medulla (RVM) and spinal dorsal horn (SDH) (see Fig. 1) [21–25].

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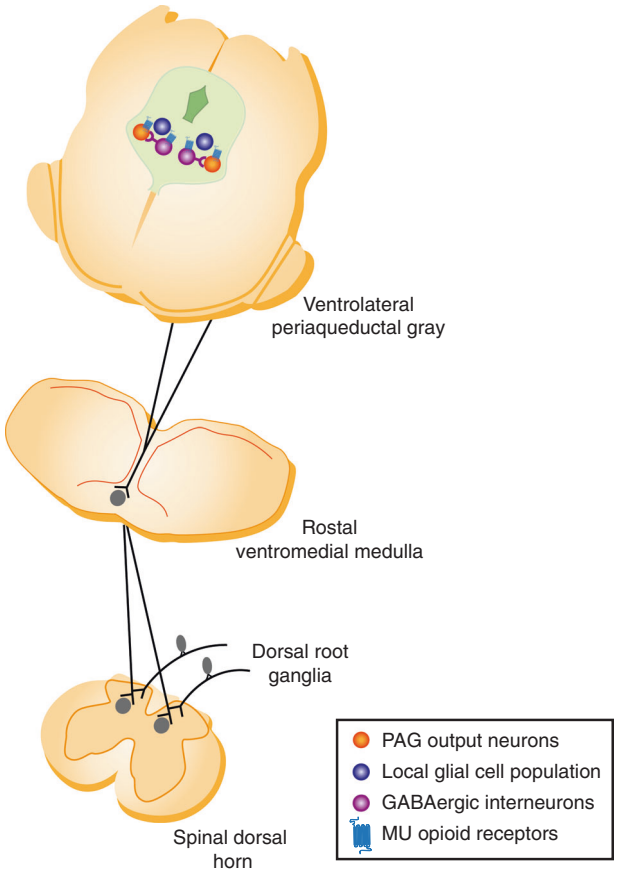

Fig. 1 A schematic of the descending inhibitory pathway for pain modulation illustrating the projections from the caudal ventrolateral column of the midbrain periaqueductal gray to the rostral ventromedial medulla in the brainstem and the dorsal horn of the spinal cord at the level of incoming stimulation from sensory neurons of the dorsal root ganglia. Also indicated are local GABAergic interneurons (purple) and PAG–RVM output neurons (orange) and a local glial cell population (blue) that also signals to the PAG–RVM output neurons. Mu opioid receptors expressed on local GABAergic interneurons and PAG–RVM output neurons are also indicated

Opioid receptors are densely populated within all three regions, and administration of opioids can elicit analgesia at each of these target sites [26–32]. Although the PAG, RVM, and spinal cord dorsal horn are all critical for opioid modulation of pain, this review focuses on the PAG as numerous lines of evidence indicate that the PAG is a primary site for opiate action. Direct administration of morphine into the PAG produces long-lasting analgesia, while site-specific blockade of opioid receptors in the PAG attenuates the analgesic effects of *systemic* morphine [29, 33, 34]. Additionally, repeated administration of morphine directly into the PAG induces tolerance [34–39]. Opioid-induced cardiorespiratory dampening has also been attributed to the effect of PAG signaling on cardiorespiratory regulation [40, 41].

SEX DIFFERENCES IN MORPHINE ANALGESIA

Both preclinical and clinical research over the last three decades has implicated sex as a biological variable influencing opioid modulation of pain. Sex differences in morphine attenuation of persistent and/or severe pain have been attributed, in part, to sexual dimorphism in the neuroanatomical, neurophysiological, and neuroimmunological aspects of the descending inhibitory circuit. Here we cohesively present the formative preclinical and clinical studies evidencing sex differences in morphine analgesia and provide support for five major factors underlying sex differences in opioid modulation of pain: (1) sex differences in the neuroanatomical organization of the PAG–RVM pathway; (2) sexually dimorphic activation of the PAG by inflammatory pain and morphine; (3) gonadal hormone-related plasticity in opioid receptor expression in the PAG; (4) sex differences in opioid metabolism and the central effects of metabolites; and (5) the role of PAG glia and neuroimmune signaling. We further argue that the current literature clearly illustrates the necessity of sex-specific research on opioid and non-opioid modulation of pain, particularly in light of the current opioid crisis in America.

Clinical studies

Clinical studies that include sex as an independent variable are limited. This is unfortunate given the significantly higher prevalence of pain reports and disorders in women (see recent review by Sorge and Totsch [42]). Of the studies that include sex as a factor, the majority demonstrate decreased analgesic efficacy of opioids in women [8, 43–47]. Indeed, one clinical study reported that females required 30% more morphine to reach the same level of analgesia as males [46]. In contrast, other studies report that the analgesic efficacy of opioids was comparable between the sexes [48–51]. Sex differences in morphine consumption (i.e., patient-controlled analgesia) have also been reported; however, importantly, morphine consumption is not a reliable indicator of morphine analgesia as women consistently experience a greater number of negative side effects associated with acute opioid consumption, including nausea, dysphoria, headache, and constipation [50, 52–54]. Perspective on the topic is also limited by the reliance on self-report in clinical studies which is remarkably subjective, due in part to cultural and societal influences [55, 56], as well as individual differences in the pain experience [57, 58]. Preclinical behavioral models of persistent and/or severe pain provide an excellent alternative method to assess sex differences in morphine action.

Preclinical studies

Preclinical research on opioid modulation of acute or persistent pain in rodents has consistently demonstrated that morphine is more efficacious in males [26, 30, 59–67]. Sex differences in morphine action are not trivial; in both persistent inflammatory pain [26, 29, 61, 65, 68–73] and visceral pain [65, 74–77], the 50% effective dose (ED_{50}) for females is approximately 2-fold higher than the ED_{50} for males. Indeed, in our recent studies using a rat model of persistent inflammatory pain, we report morphine ED_{50} values of 4.07 mg/kg in males versus 10.39 mg/kg in females [26]. Furthermore, morphine-induced hyperalgesia is also exacerbated in females [78, 79]. Sex differences in opioid analgesia are not limited to morphine; indeed, greater pain relief is observed in male rats for almost every opioid tested [80–84].

While the majority of preclinical data noted above indicate that female rats have an attenuated response to morphine compared to males, some behavioral studies report either no sex differences [85, 86] or effects in the opposite direction [87]. These discrepancies are thought to be due primarily to differences between rat and/or mouse strains, type and route of analgesic administered, the duration of pain (acute versus chronic), modality of pain examined (e.g., orofacial, visceral, inflammatory) [4], and estrous cycle status in females (discussed in a later section). A more thorough evaluation of the neuroanatomical pathways mediating opioid analgesia, specifically within the PAG and its projections to the RVM, have recently offered new evidence for an anatomical substrate contributing to sex differences in opioid analgesia [88].

NEURAL MECHANISMS UNDERLYING SEX DIFFERENCES IN ANALGESIA

The PAG–RVM pathway

One of the first demonstrations for a role of the PAG in pain modulation was conducted by D.V. Reynolds in 1969, who, in the absence of anesthesia, performed exploratory laparotomy in rats while electrically stimulating the PAG [89]. Since then, anatomical and physiological studies conducted in a variety of species have shown that the midbrain PAG plays a modulatory role in a variety of behaviors including antinociception [24, 89–92], reproduction [93–96], fear and anxiety [96], aggression [98–101], and vocalization [102, 103]. The PAG projects heavily to the RVM, which in turn projects to the dorsal horn of the spinal cord (Fig. 1) comprising a primary neural pathway mediating the effects of opioids. Morphine binds to the mu opioid receptor (MOR), an inhibitory G protein-coupled receptor, to modulate pain [26, 29, 31]. MOR activation decreases voltage-gated calcium channel conductance, in part through low voltage-activated T-type channels [104], and increases presynaptic potassium conductance [105–107] to hyperpolarize the membrane and decrease the probability of presynaptic transmitter release.

The lateral and ventrolateral columns of the PAG contain a high density of MOR, which are localized directly on PAG–RVM output neurons (45%), as well as GABAergic neurons located presynaptic to PAG–RVM output neurons (50%) [108–112]. Two primary mechanisms have been proposed to account for mu opioid-

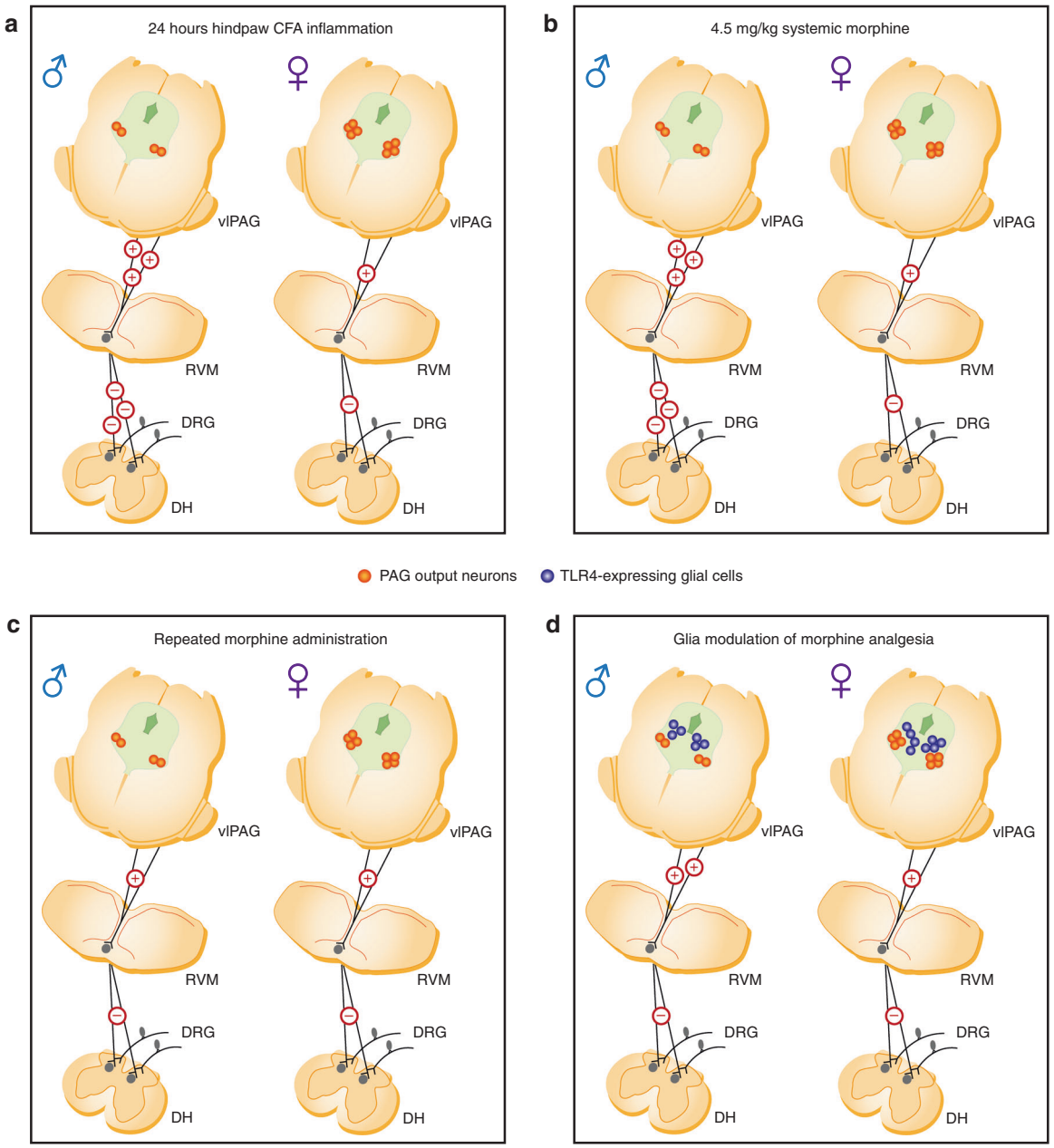

Fig. 2 Proposed model of factors contributing to sex differences in pain and morphine potency elicited by the PAG. Despite a greater number of PAG–RVM neurons (orange) in female rats, persistent inflammatory pain evoked by CFA injection into the rat hindpaw elicits greater activation of the PAG–RVM pathway in males compared to females (**a**). Similarly, systemic morphine at 4.5 mg/kg evokes greater activation of the PAG–RVM pathway in males compared to females (**b**). Repeated morphine administration leads to the development of tolerance in males, while no effect of morphine or development of tolerance was observed in females at 4.5 mg/kg (**c**). TLR4 activation of glial cells (blue) in the ventrolateral PAG of males opposes the acute effects of morphine and contributes to the development of tolerance (**d**)

induced activation of the PAG–RVM descending circuit. The *morphine disinhibition hypothesis* was proposed to account for the fact that morphine, which is inhibitory, paradoxically excites PAG–RVM output neurons [24, 113, 114]. The disinhibition hypothesis proposes that MOR + GABAergic neurons exert tonic inhibitory tone over PAG–RVM glutamatergic neurons; administration of morphine disinhibits this circuit, resulting in its activation and the inhibition of pain [106, 113, 115, 116]. Support for this hypothesis comes from in vitro studies in which MOR binding on PAG neurons reduces inhibitory postsynaptic potential frequency and decreases the probability of presynaptic GABA release [105, 106, 117]. In vivo, injection of GABA antagonists into the PAG partially mimics the effects of morphine [118] and potentiates morphine analgesia [119]. Last, selective knockdown of PAG α1G T-type channels, which are selectively expressed on intrinsic GABAergic neurons, significantly impairs morphine analgesia [119].

A second mechanism proposed to account for mu opioid-induced activation of the PAG–RVM descending circuit is a direct action of morphine on PAG–RVM output neurons. As mentioned above, MOR is also expressed on PAG–RVM output neurons [110], and opioid application to GABA-sensitive PAG–RVM neurons in a slice preparation has a predominantly inhibitory effect [120]. Both GABAergic [27] and glutamatergic [118] PAG–RVM output neurons have been identified in male rats and mice. In mice, Samineni et al. [121] reported no overlap in the distribution of glutamatergic and GABAergic neurons in the PAG, indicating two neurochemically distinct populations. Interestingly, chemogenetic activation of PAG glutamatergic neurons or inhibition of GABAergic neurons attenuates thermal pain [121]. Together, these studies indicate that opiates may act either directly on PAG–RVM neurons or indirectly via GABAergic interneurons to inhibit pain. Further adding to the complexity of morphine action in the PAG, MOR-GABAergic signaling is directly influenced by neuron–glia interactions, discussed later in this review.

Sex differences in the anatomy and physiology of the PAG–RVM pathway

The PAG and its projections to the RVM and spinal cord are sexually dimorphic in both their anatomy and physiology. Quantitatively, females have approximately twice as many output neurons in the PAG–RVM pathway as their male counterparts; however, persistent inflammatory pain or systemic administration of morphine preferentially engages this circuit in males [59, 122] (Fig. 2a, b). Males have higher levels of MOR protein and binding within the PAG [26], likely contributing to the increased analgesic efficacy of morphine observed in males. In support, site-specific lesions of PAG MOR-containing neurons in rats results in a significant rightward shift in the morphine dose–response curve in males (ED_{50} 4.07–12.55 mg/kg), making them 'female-like' in their response to morphine [26]. In contrast, lesions of PAG MOR-containing neurons in females had no effect on morphine ED_{50} (10.39–9.21 mg/kg). Together, these data indicate that anatomical and physiological differences in the PAG–RVM circuit, and in particular MOR expression, contribute to the sexually dimorphic actions of morphine. As microinjection of morphine into the RVM also results in greater antinociception in male rats in comparison to females [30], sex differences in opioidergic signaling in the RVM may also contribute to the dimorphic effects of systemic morphine.

Both pain and analgesia are modulated by emotional, motivational, and cognitive factors indicating higher-order cortical and subcortical forebrain modulation of PAG–RVM signaling. Anatomical studies in rats have demonstrated that in males, the PAG predominately receives afferents from cortical and sub-cortical sites implicated in pain and emotion, including the medial preoptic area (MPO), the central and medial nuclei of the amygdala (MeA, CeA), and the ventromedial and paraventricular hypothalamic nuclei (VMH, PVN) [123, 124]. These forebrain projections terminate with a high degree of topographical specificity within the PAG, preferentially terminating among PAG–RVM output neurons [125]. In contrast, our recent preliminary data in females suggest that MPO-PAG and CeA-PAG neurons are activated by inflammatory pain to a greater degree in female rats, while MeA-PAG neurons are more activated in male rats (unpublished data). These findings suggest that forebrain sites projecting to the PAG may also exert sexually dimorphic modulation of pain and analgesia.

Development of morphine tolerance in the PAG

Repeated administration of morphine, either systemically or directly into the ventrolateral PAG (vlPAG) results in the development of tolerance, defined as a state in which an organism no longer responds to a drug so that a higher dose is required to achieve comparable levels of analgesia [34–39]. Not surprisingly, repeated administration of systemic morphine induces tolerance to a greater degree in male versus female rats [122]. Specifically, in male rats, tolerance is accompanied by a two-fold rightward shift in the morphine dose response curve (increase in ED_{50} from 3.0 to 6.3 mg/kg). In contrast, a non-significant shift in morphine ED_{50} is observed in females (6.0 to 8.3 mg/kg). Further, the activation of PAG–RVM neurons is significantly attenuated following repeated morphine administration in males, but not females [122, 126] (Fig. 2c). This tolerance-induced reduction in PAG MOR signaling efficacy [107] is reversed when MOR coupling is enhanced via upregulated adenylate cyclase activity [127].

Chronic, but not acute, opioid administration also induces a robust neuroinflammatory response in the male rat PAG via the innate immune receptor toll-like receptor 4 (TLR4) [128, 129]. TLR4 is located on microglia, and to a lesser degree on astrocytes [130, 130]. Opioids, including morphine, bind to the glycoprotein myeloid differentiation factor-2 (MD-2) on TLR4 to initiate an inflammatory response through nuclear factor kappa B (NFκB) activation and p38 mitogen activated protein kinase (MAPK) phosphorylation [128, 132, 133]. Activation of the NFκB pathway results in the robust release of proinflammatory cytokines, including tumor necrosis factor (TNF), interleukin 1 beta (IL-1β), and interleukin 6 (IL-6). Our recent studies demonstrate that TLR4 activity within the vlPAG (Fig. 2d) directly opposes the acute effects of morphine and contribute to the development of tolerance [129, 134]. Specifically, we report that tolerance, induced by *systemic* morphine, decreases astrocytic glutamate transporter 1 and glutamate aspartate transporter (GLT-1 and GLAST, respectively), but not neuronal excitatory amino acid transporter 1 (EAACI) mRNA in the vlPAG, resulting in an increased neuroexcitatory environment. These changes in astrocytic glutamate transporter expression are dependent on vlPAG soluble TNF (solTNF) signaling as site-specific sequestration of solTNF via a dominant-negative virus or solTNF biologic reverses the decrease in GLT-1 and GLAST expression and significantly attenuates the development of tolerance [135].

Together these studies suggest that morphine binds to neuronal MOR and glial TLR4 in the vlPAG, and that concurrent activity at these receptors modulates the analgesic efficacy of morphine via two opposing mechanisms: (1) opioid binding at MOR results in hyperpolarization of GABAergic neurons and induction of opioid analgesia [106, 115]; and (2) opioid binding at glial TLR4 leads to increased vlPAG solTNF signaling that simultaneously promotes neuroinflammation and disrupts the ability of astrocytes to scavenge excess glutamate, counteracting MOR-mediated hyperpolarization of GABAergic neurons and inducing tolerance [135] (Fig. 3). The presence of MOR on microglia remains somewhat controversial [136]; however, a recent study by Corder et al. [137] using multiple histological approaches and RNA-seq of non-cultured, acutely purified adult rat spinal microglia found no evidence of MOR expression in microglia [137].

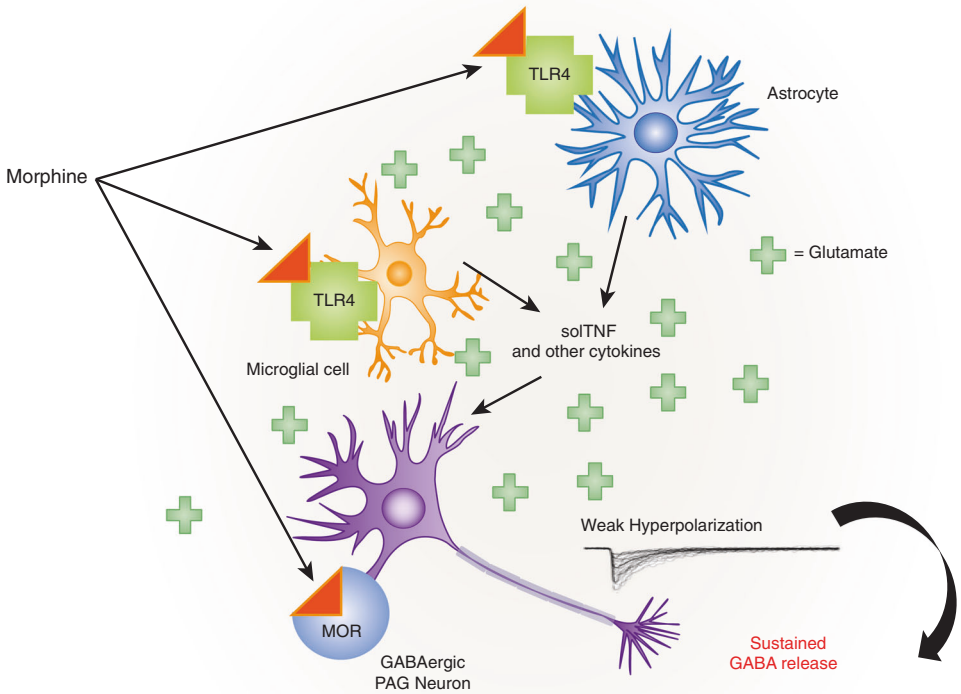

Fig. 3 Morphine binds to MOR on neurons and to TLR4 on microglia and astrocytes. While MOR binding hyperpolarizes GABAergic interneurons, TLR4 binding leads to the release of solTNF and other cytokines, which affect astrocytes by decreasing glutamate transporter proteins, and affect neurons by downregulating GABA receptors and upregulating AMPA receptors. Together, these inflammatory-induced changes increase excess glutamate and result in hyperexcitability of GABAergic interneurons, sustained GABA release, and inhibition of PAG–RVM projection neurons that dampen incoming pain signals

Although our studies clearly implicate a role for TLR4 signaling in the PAG in the development of opioid tolerance in rats, results to the contrary using TLR4 null or mutant mice have also been reported. Not surprising, TLR4 mutant mice display normal levels of antinociception following acute morphine administration; however, they also developed tolerance following repeated systemic administration [138, 139]. TLR4 null or mutant mice also displayed normal levels of opioid-induced hyperalgesia and naloxone-precipitated withdrawal following repeated morphine administration, both of which are thought to be mediated by TLR4 signaling. It is not clear why TLR4 null or mutant mice displayed tolerance and opioid-induced hyperalgesia. As stated above, opioids bind to the glycoprotein MD-2 located on TLR4 to initiate neuroinflammation and the release of TNF, and it is unknown if MD-2 signaling remains functionally intact in TLR4 null or mutant mice. In rats, although depletion of spinal microglia via the toxin saporin completely blocked morphine-induced hyperalgesia, it had no effect on tolerance [136]. Similar results were reported following intrathecal administration of the TLR4 antagonist (+)-naloxone, and together, suggest the possibility that opioid signaling at sites other than the spinal cord (e.g., PAG or primary afferent) may be more critical for the development and maintenance of morphine tolerance [137].

EFFECTS OF GONADAL HORMONES ON MORPHINE ANALGESIA

Studies in rodents indicate that sex differences in the organizational and activational effects of the gonadal hormones estradiol and testosterone influence morphine analgesia (Table 1). Male rats castrated at birth demonstrate decreased morphine potency in adulthood, while female rats masculinized at birth demonstrate greater morphine potency as adults [62, 66]. Similarly, morphine is reportedly less effective in gonadectomized adult males and more effective in ovariectomized adult females [66, 68, 73, 82, 140–142]; these effects are reversed with hormone replacement [75, 140, 143]. Moreover, the antinociceptive potency of morphine is reportedly greater during diestrus, when circulating estradiol levels are lowest [68, 70, 82, 142, 144]. In fact, it was recently reported that microinjection of morphine directly into the PAG produces less antinociception during estrus (high estradiol), and no sex difference in morphine potency was observed between diestrus females and males [29].

Table 1. Summary of evidence of the organizational and activational effects of gonadal hormones on morphine efficacy in rats

Sex	Manipulation	Reported Findings	Citations
Male	None; intact	ED_{50} 2× lower than females	[29, 60, 74, 125, 178-181]
	Gonadectomy at birth or in adulthood	Reduced morphine efficacy	[65, 66, 72, 140]
	Gonadectomy + testosterone treatment	Greater morphine efficacy than gonadectomized males	[35, 150]
	Gonadectomy + estrogen treatment	Reduced morphine efficacy compared to gonadectomized males	[67]
Female	None; cycling Diestrus Proestrus Estrus	ED_{50} 2× higher than males Efficacy similar to intact males Lower efficacy compared to diestrus Lower efficacy compared to diestrus	[60, 64, 65, 67, 72, 74, 84, 125, 141]
	Ovariectomy at birth or in adulthood	Increased morphine efficacy	[65, 66, 72, 74, 139, 140]
	Neonatal testosterone treatments	Increased morphine efficacy to male-like levels	[61, 66, 67, 81]
	Ovariectomy + estrogen treatment	Reduced morphine efficacy compared to ovariectomized females	[67, 74, 139]
	Ovariectomy + testosterone treatment	Reduced morphine efficacy compared to ovariectomized females	[67]

The PAG is a likely anatomical substrate whereby gonadal steroids influence pain and analgesia. Both androgen (AR) and estrogen receptors (ERα) have been localized in the PAG of the rat [94, 145], cat [146], golden hamster [147], guinea pig [148], and rhesus monkey [149, 150]. Male rats have a significantly greater number of AR immunoreactive neurons within the dorsomedial, lateral, and vlPAG compared to females, while PAG ERα expression is comparable between the sexes [151]. Further, approximately 30–37% of PAG–RVM output neurons in both male and female rats express AR and/or ERα, with the highest density of co-labeling observed in the lateral/ventrolateral region of PAG [151]. While the overall density of steroid receptors is similar between the sexes, fluctuating steroid levels in males and females can clearly influence this circuit. Approximately 27–50% of PAG–RVM neurons are MOR-positive [109]; given the greater density of MOR in the PAG of males than females [26], the interaction between morphine and sex hormones is likely greater in the PAG of male compared to female rats.

Several mechanisms have been proposed whereby gonadal steroids may modulate opioid-sensitive PAG–RVM output neurons, resulting in a dimorphic response to morphine. First, estradiol has been shown to uncouple the MOR from G protein-gated inwardly rectifying potassium channels [151], resulting in an attenuation of morphine-induced hyperpolarization. Second, estradiol has been shown to induce MOR internalization [152], thereby reducing available opioid binding sites on the cell membrane. Interestingly, ERα is required for estradiol-induced MOR internalization [153] supporting the hypothesis that colocalization of MOR and ERα in PAG–RVM output neurons provides a unique mechanism through which estrogens may differentially affect morphine potency in male and female rats.

FURTHER CONTRIBUTIONS OF GLIA

As discussed above, our lab as well as others [71, 133, 135, 155–163] have shown that morphine action at TLR4 initiates a neuroinflammatory response within the PAG that directly opposes the analgesic effects of morphine (although see [164]). These findings led us to test the hypothesis that the attenuated response to morphine observed in females is the result of increased microglia activation in the PAG. In these studies, male and female rats were administered morphine (or saline) and the density and phenotype of microglia (activated/reactive or non-activated/quiescent) were quantified for the vlPAG. Interestingly, we report that although there is no sex difference in the overall number or density of microglia in the vlPAG, the percentage of microglia that showed an activated phenotype (i.e., reactive) was significantly higher in females than males regardless of treatment [71]. In these studies, we also examined if the degree of microglia activation could predict an animal's response to morphine. We observed a significant relationship between morphine potency and the percentage of reactive microglia in females ($r = 0.68$), but not males ($r = 0.32$). Although acute morphine treatment did not change microglia morphology in either sex, administration of the glial TLR4 agonist lipopolysaccharide (LPS) increased the percentage of activated microglia in the vlPAG of females to a greater degree than males. This LPS-induced increase in microglia activation in females was accompanied by significantly increased proinflammatory IL-1β transcription and decreased anti-inflammatory IL-10 transcription in the PAG. We further showed that priming microglia with LPS significantly attenuated morphine analgesia in both sexes, and completely abolished the antinociceptive response to morphine in a subset of females (33%). Similarly, inhibition of vlPAG microglia with the TLR4 antagonist (+)-naloxone significantly potentiated morphine analgesia in females (ED_{50} decreased from 7.9 to 3.16 mg/kg), but not males (ED_{50} shifted from 3.04 to 5.25 mg/kg), abolishing the sex difference in opioid response. Together, these data indicate that vlPAG microglia are innately different in males and females in terms of their morphological state (both basal and following immune challenge with LPS), and further implicate TLR4 in the attenuated response to morphine observed in females.

FUTURE RESEARCH DIRECTIONS

Development of designer and alternative analgesics

Morphine, as well as other opioids that are metabolized via glucuronidation, produce a physiological response within the PAG at both MOR and TLR4 to promote and oppose opioid analgesia, respectively. Two active metabolites are produced via glucuronidation: morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G). M3G, which has a high affinity for TLR4 [133] and little to no affinity for MOR [132], induces robust microglia activation that is accompanied by cytokine release and the development of hyperalgesia [132, 165]. In contrast, M6G, which binds preferentially to MOR with little to no affinity for TLR4 [133], is the pro-analgesic metabolite of morphine [166]. Thus, M6G, and other opioids that do not activate immune cells, may produce greater analgesia and represent a favorable alternative to the commonly used immune-activating opioids.

We have recently reported that direct PAG administration of the MOR-selective metabolite M6G results in a greater analgesic response in females than morphine alone [72]. M6G analgesia was reversed with co-administration of the MOR selective antagonist (−)-naloxone, but not the TLR4 selective antagonist (+)-naloxone, indicating that this effect is MOR mediated. In contrast, intra-PAG administration of M3G significantly attenuated the analgesic effects of systemic morphine in males only, increasing the morphine ED_{50} two-fold (5.0 versus 10.3 mg/kg) and eliminating the previously observed sex difference. Together, these data implicate sex differences in morphine metabolism, and specifically the metabolite M3G, as a contributing factor in the attenuated response to morphine observed in females. More importantly, these data demonstrate that in the absence of TLR4 signaling, opioid analgesia is equally effective, if not more effective, in females as compared with males.

Historically, M6G has not been used for the treatment of clinical pain in humans, in part due to its low blood brain barrier permeability and tendency to accumulate in plasma in patients with impaired renal function [167, 168]. However, clinical trials of M6G demonstrate comparable analgesia to morphine at appropriate doses, while reducing the negative side effects typically associated with morphine, including nausea and sedation, in *both men and women* [169–172]. Future research is clearly necessary to address the relevance of treatment with M6G, as these studies may provide insight into improved treatment strategies for pain management in women.

CLINICAL IMPLICATIONS

The National Institutes of Health (NIH) recently reported new requirements for the inclusion of female subjects in NIH-funded research, as appropriate for the scientific goals of the study (Public Health Service Act sec. 492B, 42 U.S.C. sec. 289a-2). Although increasing the number of research laboratories that analyze sex-based data is clearly necessary, it is important to note that utilizing female subjects presents additional challenges that researchers need to consider in experimental design. In 2006, the Sex, Gender, and Pain Special Interest Group of the International Association for the Study of Pain (IASP) reviewed what is known about sex differences in pain and analgesia and published a seminal consensus report on the best practice guidelines for pain research including sex as an independent variable [173]. These guidelines are an excellent reference and should be consulted when designing preclinical and clinical studies utilizing female subjects.

Despite growing literature reporting sex differences in pain and morphine analgesia, the overwhelming majority of preclinical studies of pain (approximately 79%) are still conducted exclusively in males [4]. At this point, we hope it is clear that sex differences in opioid modulation of pain exist and warrant additional, comprehensive investigation into the underlying mechanisms. Building on what is currently known regarding sex differences in pain and analgesia will likely identify additional targets for the development of novel pain therapeutics; only then will we be able to advance effective pain management in both women and men.

The work referenced in this review highlights several inherent differences in how the central nervous system of males and females responds to pain and opioids. Multiple lines of evidence implicate the PAG as a key anatomical substrate underlying the observed sex differences in opioid analgesia. Based on this, we argue that morphine may not be the drug of choice for pain management in women. As both preclinical and clinical research indicate that opioids are less effective in females, it is interesting that women are more likely to be prescribed opioids at *higher doses* and for *longer periods of time* than men [174–176]. Perhaps this is illustrative of the observed lower potency of opioids in women, and mirrors preclinical studies on lower opioid potency in rodents. Further, women may be especially at risk for developing opioid addiction and overdose, which are currently being underscored in our society as an opioid crisis. These sex differences and potential vulnerabilities must be considered in pain management regimens in women until novel, non-opioid therapeutics that are equivalently effective in men and women alike, are identified.

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ADDITIONAL INFORMATION

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