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Pharmacological modulation of endogenous opioid activity to attenuate neuropathic pain in rats

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Abstract

We previously showed that spinal mGluR₁ signaling suppresses or facilitates (depending on stage of estrous cycle) analgesic responsiveness to intrathecal endomorphin 2 (EM2), a highly mu-opioid receptor (MOR)-selective endogenous opioid. Spinal EM2 antinociception is suppressed during diestrus by mGluR₁ when it is activated by membrane estrogen receptor alpha (mER α), and facilitated during proestrus when mGluR₁ is activated by glutamate. In the current study, we tested the hypothesis that in female rats subjected to spinal nerve ligation (SNL), inhibition of spinal estrogen synthesis or blockade of spinal mER α /mGluR₁ would be anti-allodynic during diestrus, whereas during proestrus mGluR₁ blockade would worsen the mechanical allodynia. As postulated, following SNL, aromatase inhibition or mER α /mGluR₁ blockade during diestrus markedly lessened mechanical allodynia. This was observed only on the paw ipsilateral to SNL and was eliminated by naloxone, implicating endogenous opioid mediation. In contrast, during proestrus, mGluR₁ blockade worsened SNL-induced mechanical allodynia of the ipsilateral paw. Findings suggest menstrual cycle stage-specific drug targets for and the putative clinical utility of harnessing endogenous opioids for chronic pain management in women, as well as the value of, if not the necessity for, considering menstrual cycle stage in clinical trials thereof.

Keywords

endogenous opioids; neuropathic pain; estrogenic signaling; estrous cycle; spinal cord

Introduction

Endomorphins (EMs) are widely considered to be the endogenous ligands for the mu-opioid receptor (MOR), the predominant opioid receptor mediating antinociception. Central administration of EM1 (Tyr-Pro-Trp-Phe-NH₂) and EM2 (Tyr-Pro-Phe-Phe-NH₂) produce potent MOR-mediated antinociception.^{57,60,70} We previously reported that the magnitude of antinociception elicited by the spinal application of EM2, varies across the rat estrous cycle,

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minimal during diestrus but robust, comparable to that of male rats, during proestrus.⁴⁵ During diestrus, spinal EM2/MOR antinociception is suppressed by spinally synthesized estrogens via their stimulation of spinal membrane estrogen receptors (mERs) that signal via metabotropic glutamate receptor 1 (mGluR₁).⁴⁷ The striking emergence of robust spinal EM2 antinociception during proestrus requires not only the loss of mER-mGluR₁ suppression, but also the emergence of signaling not present during diestrus. This includes increased release of spinal dynorphin, activation of spinal kappa-opioid receptors (KOR), and a switch in the endogenous agonist of mGluR₁ from mER α in diestrus to glutamate in proestrus.⁴⁶

It is likely that signaling mechanisms regulating analgesic effectiveness of intrathecally applied EM2 also pertain to endogenous EM2 antinociception. An anatomical organization is present in spinal cord that enables endogenous interactions among presumed regulatory components of intrathecal (i.t.) EM2 antinociception that parallel those that occur following the spinal application of EM2. Spinal neurons coexpressing MOR, mER α , mGluR₁, and aromatase (estrogen synthase) are apposed by EM2 varicosities.⁴⁷ This provides an anatomical basis for physiologic modulation of endogenous spinal EM2 analgesic responsiveness by mER α -mGluR₁ signaling. Additionally, spinal dynorphin-expressing neurons that coexpress mGluR₁ and ER α are apposed by terminals expressing vesicular glutamate transporters (glutamatergic terminals).⁴⁶ This cellular organization not only provides basis for cycling between ER α -activated and glutamate-activated mGluR₁ signaling over the estrous cycle, but also enables glutamate modulation of spinal dynorphin release, thereby coordinating glutamate activation of mGluR₁ with dynorphin release, both of which are prerequisites for spinal EM2 antinociception during proestrus.⁴⁶

The identification of signaling molecules that gate analgesic responsiveness to spinal EM2 suggests them to be potential targets for tapping into the spinal EM2 analgesic system for pain management. However, under resting physiological conditions, in the absence of acute or chronic pain, endogenous opioid systems are quiescent, i.e., naloxone does not alter basal pain thresholds in laboratory animals^{4,24} and humans.^{16,25} Endogenous opioid systems do, however, influence nociceptive response thresholds when activated by noxious stimuli, e.g. acute or chronic pain.^{30,41,52,54,68} Accordingly, we tested the hypothesis that i.t. treatments that unleash the analgesic properties of spinal EM2 during diestrus will also be anti-allodynic, in the absence of exogenous opioids, in a spinal nerve ligation (SNL) chronic pain model during diestrus. We also tested the hypothesis that spinal treatments that impair spinal EM2 antinociception during proestrus⁴⁶ will exacerbate SNL-induced mechanical allodynia during this estrous cycle stage.

Our findings reveal that compromised spinal estrogenic and mGluR₁ activity, which unleashes antinociceptive responsiveness to i.t. EM2 in intact diestrous rats^{46,47} reduced spinal opioid antiallodynia in spinal nerve ligated rats during diestrus. Conversely, compromised mGluR₁ signaling, which minimizes i.t. EM2 antinociception during proestrus⁴⁶, exacerbated SNL-induced mechanical allodynia in proestrous rats. These findings suggest menstrual cycle stage-specific drug targets for and the putative clinical utility of harnessing endogenous opioids for chronic pain management in women. Additionally, results underscore the value of, if not the imperative for, considering menstrual

cycle stage when developing novel pain medications as well as when designing clinical trials.

Methods

Animals and Housing

Experiments used female Sprague-Dawley rats (Charles River Laboratories, Kingston, NY; 225–275 g) maintained in a controlled environment on a 12-hour light/dark cycle. Food and water were available *ad libitum*. All experimental procedures were reviewed and approved by our Institutional Animal Care and Use Committee and conform to applicable national/international guidelines. Animals were randomly assigned to experimental and control groups. Each animal was used only once. In total, there were nine experimental groups and two vehicle (DMSO or saline) groups. The experimental groups included: ER α blockade, mGluR $_1$ blockade, aromatase inhibition (in proestrus and diestrus), each in combination with naloxone pretreatment in diestrus. Group size was guided by power analysis and is indicated in relevant sections of Results.

Determination of stage of estrous cycle

Estrous cycle stage was determined by histology of vaginal smears. Proestrus or diestrus showed a predominance of large round nucleated cells or small leukocytes, respectively. Potential disruptions of the estrous cycle resulting from surgery did not confound data interpretation because stage of cycle was defined by vaginal smear histology rather than predictions assuming regularity of cycling. Experiments were performed following resumption of normal cycling.

Spinal nerve ligation

An incision was made above the lumbar spine and the left transverse process of vertebra L6 was exposed under isoflurane anesthesia (2.5%). The left L5 spinal nerve was tightly ligated with silk thread No. 6 and cut distal to the ligation.^{6,33,34,39,43,56} Peripheral neuropathic pain emerges in the ipsilateral hind paws, manifested as mechanical allodynia,^{6,43,56} within 24 h, which remains stable for months (mechanical allodynic thresholds are unaffected on the contralateral side). General behavior of the rats was monitored before and after the surgery. Rats showing difficulty elevating the hind paw ipsilateral to SNL were excluded from the study.

Implantation of Intrathecal Cannulas

For spinal drug delivery, a permanent indwelling cannula was inserted into the lumbar spinal cord subarachnoid space as described previously.^{46,47} Spinal cannula implantation was performed concomitant with SNL. In brief, animals were anesthetized as described above, and an 8.0 cm PE-10 catheter (Becton, Dickinson and Company, Franklin Lanes, NJ) was inserted through the atlantooccipital membrane into the spinal subarachnoid space. The cephalic portion of the catheter was secured in place and externalized through the skin on the dorsal side of the neck, where it was relatively inaccessible to the paws. Upon gross inspection, all animals appeared to be free of infection. The righting reflex and the inclined

plane test were used to assess motoric integrity; any animals showing motor impairment following surgery were excluded.

Intrathecal Administration of Drugs

1,3-Bis (4-hydroxyphenyl)-4-methyl-5-[4-(2-piperidinylethoxy) phenol]-1H-pyrazole dihydrochloride [MPP, an ER α -selective antagonist, obtained from Tocris (Ellisville, MO)] and fadrozole [an aromatase inhibitor obtained from Sigma (St Louis, MO)] were each prepared in 3 μ L dimethyl sulfoxide (DMSO). 6-amino-N-cyclohexyl-N,3-dimethylthiazolo [3,2-a] benzimidazole-2-carboxamide hydrochloride [YM298198, an mGluR $_1$ antagonist, obtained from Tocris (Ellisville, MO)] and naloxone (opioid receptor antagonist, obtained from NIDA) were each prepared in 5 μ L saline. Drugs were applied to the spinal cord subarachnoid space over a 60-second period via the indwelling i.t. cannula. Complete delivery was ensured by flushing the cannula with an additional 10 μ L of saline. Importantly, a number of studies have indicated the absence of significant supraspinal diffusion/effects of i.t. injectate even 60 minutes following i.t. injection.^{1,29,38} I.t. doses of MPP (10 nmol), YM298198 (25 nmol), fadrozole (2.5 nmol) and naloxone (25 nmol) were based on previous studies^{8,11,18,35,44,46,47,62} in order to maintain parallelism of intrathecal treatments used in the current report and its two antecedent studies.^{46,47}

Quantification of Mechanical Hypersensitivity/Allodynia

Mechanical allodynia was quantified by measuring paw withdrawal thresholds of both ipsilateral and contralateral hind paws in response to the application of von Frey hairs, as described by Chaplan.¹⁰ In brief, rats were placed on a wire mesh surface, covered by an inverted plastic cage and allowed to habituate for 15 min. A hand-held probe containing a rigid filament was applied to the plantar surface of the hindpaw with increasing force. The applied pressure (g) that elicited paw withdrawal was automatically recorded using Electronic von Frey Anesthesiometer (IITC Life Science, Woodland Hills, CA). Withdrawal thresholds of the paw ipsilateral to SNL were compared with the contralateral paw. All testing was performed blind to cycle stage and treatment.

Data Analysis

Two-way ANOVA was used to compare paw withdrawal threshold (contralateral and ipsilateral to SNL) by cycle stage (proestrous, diestrous). Two-way repeated measure ANOVA was used to compare treatment by time effects among groups. Bonferroni post hoc test identified specific groups between which and times at which significant effects were observed. $P < 0.05$ was considered significant. Data are expressed as the mean $\pm$ standard error of the mean (SEM).

Results

SNL induced mechanical allodynia during diestrus and proestrus

As expected,^{6,43,56} during either proestrus or diestrus, SNL increased mechanical hypersensitivity (approximately 3-fold; two-way ANOVA; $F_{1,152}=373.1$, $p<0.0001$ for mechanical sensitivity of ipsilateral vs. contralateral hind paws, $n=18$ for proestrus and 60 for diestrus; Fig. 1). SNL failed to alter mechanical sensitivity on the side contralateral to the

lesion (data from surgically naïve rats not shown). Additionally, the two-way ANOVA also revealed a small but significant difference between proestrous and diestrous groups ($F_{1,152}=4.244$, $p=0.041$). This indicated that rats are more susceptible to develop greater surgically-induced mechanical allodynia during diestrus than proestrus.

Blockade of spinal mER α or inhibition of spinal aromatase produced a spinal opioid-mediated decrement in SNL-induced allodynia during diestrus

Since during diestrous, spinal EM2 antinociception can be unmasked by comprising spinal estrogenic activity⁴⁷, we hypothesized that doing so would also substantially attenuate SNL-induced mechanical allodynia (by augmenting endogenous EM2 antinociception). This was first tested by determining the influence of spinal mER α blockade [via i.t. MPP (10 nmol)^{44,47} on SNL-induced mechanical allodynia]. I.t. MPP significantly increased (~160%) von Frey tactile response thresholds of the paw ipsilateral (but not contralateral) to SNL in diestrous rats, which was abolished by i.t. naloxone (25 nmol, 30 min prior to i.t. MPP), i.e., i.t. MPP was no longer antiallodynic following naloxone pretreatment.

Two-way repeated measure ANOVA revealed a significant difference in treatment effect among MPP, naloxone + MPP and DMSO vehicle (control) groups ($F_{2,18}=12.44$, $p<0.0004$, $n=6-9$) (Fig. 2, left panel). Additionally, Bonferroni post hoc comparisons revealed significant differences between the MPP group vs. the vehicle control and naloxone + MPP groups ($p<0.05$ for 5–20 min time points for both comparisons; specific p value for each time point is shown in Fig. 2). Notably, there was no difference between naloxone + MPP group and the vehicle control group ($p>0.05$). This indicated that spinal naloxone abolished the ability of MPP to diminish mechanical allodynia during diestrus. Strikingly, i.t. MPP did not alter mechanical allodynia of the contralateral paw ($F_{1,12}=0.19$, $p=0.670$ in comparison to control group, $n=6$; data not shown). Since proestrus is the cycle stage during which MPP does not alter the robust spinal EM2 antinociception manifest by intact female rats (without SNL)⁴⁷, it was not surprising that i.t. MPP also failed to alter allodynic thresholds in spinal nerve ligated rats during proestrus ($F_{1,11}=1.44$, $p=0.249$, $n=6$, data not shown).

Analogous to effects of spinal mER α blockade, i.t. treatment of spinal nerve ligated rats with the aromatase inhibitor fadrozole (2.5 nmol)^{44,47} (to minimize the spinal synthesis of estrogens) also significantly decreased mechanical allodynia of the paw ipsilateral (but not contralateral) to SNL during diestrus. This effect was abolished by a 30 min i.t. pretreatment with naloxone. Two-way repeated measure ANOVA revealed a significant difference in treatment effect among groups [fadrozole, naloxone + fadrozole, and saline vehicle (control) groups; $F_{2,17}=11.25$, $p=0.0008$, $n=6-8$; Fig. 2, right panel]. Bonferroni post hoc comparisons revealed significant differences between the fadrozole group and the vehicle control, as well as the naloxone + fadrozole group ($p<0.05$ for 5–30 min time points for both comparisons; specific p value for each time point shown in Fig. 2). Importantly, there was no difference between the naloxone + fadrozole group and the vehicle control group ($p>0.05$), indicating that naloxone abolished the anti-allodynic effect of i.t. fadrozole. As was observed for i.t. MPP, administration of i.t. fadrozole alone did not alter mechanical allodynia of the contralateral paw ($F_{1,12}=0.95$, $p=0.349$ in comparison to control group, $n=6$; data not shown). Since we previously reported⁴⁷ that i.t. fadrozole failed to alter i.t. EM2 analgesic

responsiveness in intact proestrous rats⁴⁷ (i.e., there is a loss of spinal estrogenic modulation of i.t. EM2 antinociception in intact rats at this estrous cycle stage), it was not surprising that i.t. fadrozole also failed to affect allodynic response thresholds in spinal nerve ligated rats during proestrus ($F_{1,10}=0.732$, $p=0.412$, $n=6$, data not shown).

Blockade of spinal mGluR₁ produced opposing modulation of SNL-induced mechanical allodynia during diestrus and proestrus

Similar to effects of inhibiting spinal aromatase or blocking spinal mER α , blockade of spinal mGluR₁ during diestrus [via i.t. YM298198 (25 nmol)⁴⁶] also significantly decreased SNL-induced allodynia, increasing response threshold by ~2 fold. This effect (not observed on the contralateral side) was eliminated by a 30 min i.t. pretreatment with naloxone. Two-way repeated measure ANOVA revealed significant differences in treatment effect among YM298198, naloxone + YM298198, and saline vehicle (control) groups ($F_{2,18}=10.44$, $p=0.001$, $n=6-8$; Fig. 3). Additionally, Bonferroni post hoc comparisons indicated significant differences between the YM298198 vs. the vehicle control as well as vs. naloxone + YM298198 groups ($p<0.05$ for 10–25 min time points for both comparisons; specific p value for each time point shown in Fig. 3). In contrast, there was no difference between naloxone + YM298198 and the vehicle control groups ($p>0.05$). As was observed for i.t. MPP and fadrozole, i.t. YM298198 alone did not alter mechanical allodynia of the contralateral paw ($F_{1,12}=0.97$, $p=0.345$ in comparison to control group, $n=6$; data not shown). Since we previously reported that mGluR₁ blockade *diminished* i.t. EM2 analgesic responsiveness in intact proestrous rats⁴⁶, it was not surprising that i.t. YM298198 *exacerbated* SNL-induced mechanical allodynia during this estrous cycle stage ($F_{1,10}=6.52$, $p=0.029$, $n=6$; Fig. 4).

Discussion

Our findings identify spinal targets for developing drugs to harness endogenous spinal opioid antinociception. We had previously identified several signaling molecules that modulated analgesic responsiveness to intrathecally applied EM2, which we postulated also pertain to modulating *endogenous* spinal EM2 antinociception.^{46,47} These antecedent reports notwithstanding, the relevance of spinal aromatase, mER α and mGluR₁ to endogenous opioid antinociception and the relevance this opioid antinociception to experimentally induced mechanical allodynia remained hypothetical. Here we demonstrate a striking parallelism between the ability of specific i.t. treatments to alter spinal EM2 analgesic responsiveness in intact rats and the ability of such i.t. treatments to alter mechanical allodynia produced by SNL.

The i.t. treatments employed in this study that lessened SNL-induced mechanical allodynia during diestrus (blockade of spinal mER α /mGluR₁, inhibition of spinally synthesized estrogens) all unmask i.t. EM2 analgesic responsiveness in intact diestrous rats.⁴⁷ Similarly, the i.t. treatment that worsened SNL-induced allodynia during proestrus (mGluR₁ blockade) substantially attenuates i.t. EM2 analgesic responsiveness during proestrus.⁴⁶ These data strongly suggest that the functional state of the endogenous spinal EM2 system, and likely other endogenous opioid systems, can significantly influence the severity of mechanical

allodynia resulting from SNL (and perhaps other chronic pain conditions). This inference is bolstered by our current demonstration that spinal opioid receptor blockade eliminated the anti-allodynic effects of the i.t. treatments.

It is possible that elimination by naloxone of the anti-allodynic consequences of minimizing either spinal estrogenic or mGluR₁ signaling during diestrus resulted from generalized pronociceptive/pro-allodynic effects of opioid receptor blockade, unrelated to the ability of i.t. treatments to disinhibit endogenous spinal opioid activity. Two considerations argue against this. First, i.t. naloxone failed to alter tactile responsiveness of the contralateral paw, as would likely have resulted from generalized consequences of opioid receptor blockade. Second, 30 min following i.t. naloxone (pretreatment period prior to i.t. MPP, fadrozole or YM298198), mechanical responsiveness of the ipsilateral paw was not different from that measured prior to treatment. Thus, the most parsimonious interpretation is that the anti-allodynic effects of compromising estrogenic or mGluR₁ signaling during diestrus resulted from augmented endogenous spinal opioid activity (presumably, at least in part, via EM2).

Since estrogens can be both pronociceptive^{7,27,28,42,48,58} as well as antinociceptive,^{2,9,17,23,36,37,49,55,59} it is possible that the anti-allodynic effect of spinal ER α blockade or spinal aromatase inhibition in spinal nerve ligated diestrous rats resulted from the loss of ER-mediated mechanical hypersensitivity, unrelated to endogenous spinal opioid antinociception. Several considerations argue against this: (1) the loss of ER-mediated mechanical hypersensitivity, irrespective of its underlying mechanism (e.g., upregulation of NMDA expression,¹⁴ or TRPV1 channels⁶⁷, enhancement of inflammation⁶⁷) would not be expected to be blocked by naloxone, as was found to be the case, and (2) i.t. MPP was not anti-allodynic on the contralateral side during diestrus and the ipsilateral/contralateral sides during proestrus.

Analogous considerations also argue against the possibility that the anti-allodynic effect of spinal mGluR₁ blockade in spinal nerve ligated diestrous rats resulted from the loss of pronociceptive influences of spinal mGluR₁,^{22,31,50,53,69} independent of unleashing spinal opioid antinociception. In this regard, it is relevant to note that pronociceptive influences of mGluR₁ are usually attributed to its activation by glutamate.^{19,66} In contrast, the unmasking of i.t. EM2 antinociception during diestrus by YM298198⁴⁷ (and presumably its anti-allodynic effect observed in this study) results from antagonizing mGluR₁ that is activated by mER α .⁴⁷ Furthermore, during proestrus, mGluR₁ blockade worsened mechanical allodynia of the ipsilateral hind paw (and diminished i.t. EM2 antinociception⁴⁶). These findings argue that the currently observed antiallodynic effect of blocking spinal mGluR₁ in spinal nerve ligated rats did not result from the loss of pronociceptive mGluR₁ signaling during diestrus, but rather the disinhibition of spinal opioid (EM2) antinociception.

Although it is well documented that activity of ionotropic glutamate receptors can influence responsiveness to opioids (e.g., NMDA antagonists attenuate the development of opioid tolerance and dependence,^{5,63,72} and restore the analgesic efficacy of morphine in neuropathic rats^{12,51}), the modulatory influence of mGluRs on opioid responsiveness have been much less studied (notwithstanding the report that mGluR₁ knockdown can restore morphine analgesic efficacy in neuropathic rats²²). Our previous study^{46,47} demonstrated

that endogenous mGluR₁ activity is an essential component of the mechanisms that suppress and facilitate the spinal opioid antinociception elicited by the intrathecal application of (exogenous) EM2. To the best of our knowledge, the current report is the first to demonstrate that endogenous mGluR₁ activity is also a critical component of the spinal mechanisms that suppress as well as facilitates (depending on the physiological state) *endogenous* spinal opioid anti-allodynia.

Multiple nociceptive conditions have been used to demonstrate anti-hyperalgesic and antiallodynic effects of mGluR₁ blockade. These include carrageenan-induced inflammation and noxious thermal stimuli,^{22,69} colonic distension,³¹ and neuropathic pain involving thermal hypersensitivity, and mechanical allodynia.^{20,22} Overall, an abundance of evidence indicates that mGluR₁ modulates nociceptive processing at various levels in the nervous system and is involved in central sensitization associated with chronic pain.^{22,32,50,64} Notably, these studies did not investigate the contribution of *endogenous* opioids to the pain relieving consequences of blocking mGluR₁, as is demonstrated in the current study. Generalization of the ability of mGluR₁ blockade to harness endogenous opioid antinociception across pain states remains to be established.

Antinociceptive influences of mGluR₁ activity are not always equivalently manifest across nociceptive conditions; e.g., knockdown of spinal mGluR₁ does not affect pain thresholds in normal rats⁷¹. In contrast, spinal mGluR₁ knockdown does attenuate inflammation-²¹ and nerve injury-induced²² mechanical hypersensitivity and allodynia. This divergence and plasticity of contributions of mGluR₁ signaling to nociception is underscored by the current demonstration that mGluR₁ blockade during proestrus significantly *worsened* SNL-induced mechanical allodynia. Interestingly, whereas prior reports demonstrate variable mGluR₁ nociceptive contributions across different pain modalities, the current study revealed qualitatively divergent consequences of mGluR₁ signaling on nociception that were dependent on physiological state within the same pain model.

Intriguingly, the spinal opioid-mediated anti-allodynic effects of fadrozole, MPP, and YM298198 were observed exclusively in the ipsilateral paw, contrary to the expectation of bilateral involvement had endogenous spinal opioid activity been augmented throughout the spinal cord. This suggests that nociceptive challenge (e.g., SNL) can selectively activate endogenous opioid activity within specific spinal neuronal circuits. This is consonant with reports that dermatomedirected anticipation of pain relief can induce activation of endogenous opioid antinociception that is exclusively directed at the body part that is targeted by expectation.³

There are additional precedents for the potential clinical utility of harnessing the activity of endogenous opioids for pain management. An exemplar of this is the endogenous opioid mediation of placebo-induced antinociception, demonstrations of which include: (a) opioid receptor blockade eliminates placebo-induced reduction of postoperative dental pain⁴⁰ and enhances clinical nociception;⁴¹ (b) expectation of pain relief activates MOR in human brain, which correlates with reduced pain intensity as well as its sensory and affective qualities;⁷³ (c) placebo analgesia against noxious thermal hyperalgesia largely results from the potentiation of endogenous opioid release in key brain regions;⁶⁵ (d) analgesic effects

reported with transcranial direct current stimulation results in part, from enhanced recruitment of MORs;¹⁵ (e) tissue injury leads to constitutive endogenous activation of MOR that can repress spinal nociception;¹³ (f) repetitive transcranial magnetic stimulation produces analgesic effects that derive from endogenous opioidergic activity.⁶¹

Although the above examples of opioid-mediated placebo analgesia underscore the therapeutic potential of incorporating strategies for harnessing endogenous opioids into paradigms for pain management, they do not provide pharmacological targets for effectively doing so. In the current study, we selected spinal mER α , aromatase and mGluR₁ as potential targets for harnessing endogenous opioid antinociception following SNL. Although confirming opioid mediation of the anti-allodynia that ensues following their compromised functionality, we have not unequivocally identified the specific endogenous opioid(s) involved. However, mediation by endogenous EM2, at least in part, is strongly suggested by the parallelism between i.t. treatments that decrease mechanical allodynia in spinal nerve ligated diestrus rats (current study) and unmask i.t. EM2 antinociception in intact diestrus rats.⁴⁷ Furthermore, the ability of mGluR₁ blockade to both eliminate i.t. EM2 antinociception in intact proestrous rats⁴⁶ and exacerbate mechanical allodynia in proestrous spinal nerve ligated rats (current study) further suggests the relevance of the ebb and flow of endogenous spinal EM2 antinociception to our ability to pharmacologically manipulate the magnitude of SNL-induced mechanical allodynia.

The relevance of current findings to nociception/opioid antinociception in women remains to be established. Of note, the peak severity of many highly prevalent chronic pain conditions in women (e.g., fibromyalgia and migraine) occurs around the time of menses,²⁶ which corresponds to diestrus in rats. This suggests that spinal application of ER α blockers or aromatase inhibitors (many of which are FDA approved), and mGluR₁ antagonists could be useful in managing some of the chronic pain syndromes experienced by women, which remain incompletely understood and poorly managed.

A pain management strategy that incorporates tapping into endogenous opioid systems would greatly lessen the use of exogenous opioids for chronic pain management. This is particularly important in light of the current prescription opioid abuse epidemic. Furthermore, the present demonstration of stage of estrous cycle-dependent qualitative divergence in the contribution of spinal signaling molecules to mechanical allodynia (e.g., mGluR₁ – worsening it during diestrus but lessening it during proestrus) has major implications for analgesic drug development, emphasizing the need to not only include women, but also to take stage of menstrual cycle into consideration when investigating efficacy of new analgesics. One wonders how many promising analgesics have been eliminated by clinical trials that failed to consider menstrual cycle stage as a critical biological variable.

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Highlights

- Treatments that modulate spinal endomorphin 2 analgesia also modulate allodynia
- Decreasing spinal estrogenic or mGluR₁ signaling lessens allodynia in diestrous rats
- Anti-allodynic effects of reducing estrogenic or mGluR₁ signaling are opioid mediated
- Endogenous opioid systems can be pharmacologically activated to treat chronic pain

Perspective:

Intrathecal treatments that enhance spinal EM2 analgesic responsiveness under basal conditions lessen mechanical allodynia in a chronic pain model. Findings provide a foundation for developing drugs that harness endogenous opioid antinociception for chronic pain relief, lessening the need for exogenous opioids and thus prescription opioid abuse.

Figure 1.

SNL induces allodynia in both proestrus and diestrus. Left SNL of L5 was performed in female rats. One week after surgery, identification of cycle stage was initiated. Mechanical allodynic thresholds were determined using an Electronic von Frey Anesthesiometer. The right contralateral (Contra) paw was used as control. Data show basal pain thresholds (obtained from rats used in all experiments), which are presented as mean + SEM. N=60 and 18 for diestrus (D) and proestrus (P), respectively. Results not only show a substantial increase in mechanical allodynia of the ipsilateral (Ipsi) paw of both D and P, but also that D is more susceptible than P to develop experimental mechanical allodynia. ****, $p < 0.0001$ in comparison to the corresponding Contra paw; #, $p < 0.05$ for the comparison between P and D.

Figure 2.

Blockade of spinal ER α as well as aromatase inhibition induces anti-allodynia in spinal nerve ligated rats during diestrous. Mechanical allodynic thresholds were determined before and after i.t. treatment at the indicated time points using an Electronic von Frey Anesthesiometer. I.t. application of either the ER α blocker (MPP, 10 nmol; left panel; n=9) or the aromatase inhibitor fadrozole (FAD, 2.5 nmol; right panel; n=8) decreases mechanical allodynia, which is blocked by a 30 min pretreatment with i.t. naloxone (pre-NX, 25 nmol; n=6 for both MPP and FAD groups). Data are presented as mean $\pm$ SEM change in paw withdrawal threshold to mechanical stimulation. * or #, $p < 0.05$; ** or ##, $p < 0.01$; *** or ###, $p < 0.001$ in comparison to naloxone + MPP and vehicle (Veh: DMSO for MPP and saline for FAD) treatment groups, respectively.

Figure 3.

Blockade of spinal mGluR₁ in spinal nerve ligated rats produces anti-allodynia during diestrus. Mechanical allodynia was quantified before and after i.t. treatment at the indicated time points using an Electronic von Frey Anesthesiometer. I.t. application of the mGluR₁ blocker YM298198 (YM, 25 nmol; n=8) produces anti-allodynia that is blocked by a 30 min pretreatment with i.t. naloxone (pre-NX, 25 nmol; n=6). Data are presented as mean $\pm$ SEM change in paw withdrawal threshold to mechanical stimulation. * or #, $p < 0.05$; ** or ##, $p < 0.01$; **** or ####, $p < 0.0001$ in comparison to naloxone + YM and vehicle (Veh=saline) treatment groups, respectively.

Figure 4.

Blockade of spinal mGluR₁ in spinal nerve ligated rats enhances allodynia during proestrus. Mechanical allodynia was quantified before and after i.t. treatment at the indicated time points using an Electronic von Frey Anesthesiometer. I.t. application of the mGluR₁ blocker YM298198 (YM, 25 nmol; n=6) exacerbated mechanical allodynia. Data are presented as mean $\pm$ SEM change in paw withdrawal threshold to mechanical stimulation. *, $p < 0.05$ in comparison to vehicle (Veh=saline) treatment group.