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Arbiters of endogenous opioid analgesia: role of CNS estrogenic and glutamatergic systems

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Abstract

Nociception and opioid antinociception in females are pliable processes, varying qualitatively and quantitatively over the reproductive cycle. Spinal estrogenic signaling via membrane estrogen receptors (mERs), in combination with multiple other signaling molecules [spinal dynorphin, kappa-opioid receptors (KOR), glutamate and metabotropic glutamate receptor 1 ($mGluR_1$)], appears to function as a master coordinator, parsing functionality between pronociception and antinociception. This provides a window into pharmacologically accessing intrinsic opioid analgesic/anti-allodynic systems. In diestrus, membrane estrogen receptor alpha (mERα) signals via $mGluR_1$ to suppress spinal endomorphin 2 (EM2) analgesia. Strikingly, in the absence of exogenous opioids, interfering with this suppression in a chronic pain model elicits opioid anti-allodynia, revealing contributions of endogenous opioid(s). In proestrus, robust spinal EM2 analgesia is manifest but this requires spinal dynorphin/KOR and glutamate-activated $mGluR_1$. Furthermore, spinal $mGluR_1$ blockade in a proestrus chronic pain animal (eliminating spinal EM2 analgesia) exacerbates mechanical allodynia, revealing tempering by endogenous opioid(s). A complex containing mu-opioid receptor, KOR, aromatase, mGluRs, and mERα are foundational to eliciting endogenous opioid anti-allodynia. Aromatase-mERα oligomers are also plentiful, in a central nervous system region-specific fashion. These can be independently regulated and allow estrogens to act intracellularly within the same signaling complex in which they are synthesized, explaining asynchronous relationships between circulating estrogens and central nervous system estrogen functionalities. Observations with EM2 highlight the translational relevance of extensively characterizing exogenous responsiveness to endogenous opioids and the neuronal circuits that mediate them along with the multiplicity of estrogenic systems that concomitantly function in phase and out-of-phase with the reproductive cycle.

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INTRODUCTION

Despite the discovery of endorphins dating back to the 1980s, little is known regarding the regulatory parameters governing the role of endogenous opioids in endogenous pain management. Thus, it is not surprising that the pharmacopeias contain virtually no drugs designed to act indirectly on pain control via their activation of endorphins. Until such time as the null hypothesis is proven, ie, that in situ, naturally occurring endorphins have little or no role in endogenous nociception and/or allodynia, the naturally occurring pool of opioids must be viewed as an as of yet untapped reservoir of analgesic/antiallodynic potential.

Effectively tapping into endogenous opioids for pain relief requires a somewhat detailed understanding of parameters that influence the in situ manifestation of the analgesic/antiallodynic functionality of endorphins, enabling them to act within a time frame commensurate with currently available narcotic and alternative pain-relieving drugs. Among the factors that have emerged to be relevant, central nervous system (CNS) estrogens and their receptors hold center stage along with glutamate, metabotropic glutamate receptors (mGluRs), dynorphin and the kappa-opioid receptor (KOR).

Estrogens:

Estrogenic signaling is a major determinant of opioid functionality. Pain and its relief by opioids vary over the reproductive cycle in both laboratory animals as well as humans.[1–7] In fact, there is a prominent divergence in the employment of the highly selective endogenous μ-opioid receptor (MOR) ligand endomorphin 2 (EM2) in nociceptive processing in males vs. females, that tracks the estrous cycle, oscillating between analgesically active and inactive states (in proestrous and diestrous females, respectively). This is in stark contrast to stable analgesic responsiveness to intrathecal EM2 in males.[7] Adding to this complexity, the predominant estrogen 17-β-estradiol (E2), is pronociceptive,[8–13] and antinociceptive (analgesic),[14–22] both occurring via multiple mechanisms.

Estrogens can act via classical nuclear estrogen receptors (ERs) that function as estrogen-activated transcription factors,[23] or via membrane estrogen receptors (mERs) (that passage to the plasma membrane from the nucleus), eg, estrogen receptor α (ERα)/estrogen receptor β (ERβ)[24,25] and, a G protein-coupled ER (GPR30).[26–29] These mERs concentrate in caveolae subsequent to being palmitoylated, and activate membrane signaling sequelae.[30–33] Armed with this knowledge, one can hypothesize a number of mechanisms that could mediate the fluctuation of the magnitude of intrathecal EM2 analgesia in proestrus and diestrus. These include: (1) augmented facilitation during proestrus, when plasma E2 levels are elevated, (2) alterations in the balance between estrogenic pronociception and antinociception, (3) disengagement of spinal ERs from EM2 analgesic responsiveness, etc., each of which would have differing implied therapeutic implications. Unexpectedly, however, an opposite relationship exists between estrogenic regulation of spinal EM2 analgesic responsiveness and peripheral levels of estrogens. This relationship is associated with stage of estrous cycle-temporally correlated interactions among components of a recently discovered signaling complex that is comprised of an oligomer containing MOR, KOR, mGluRs, ERα, GPR30 and aromatase (aka estrogen synthase).

Paradoxically, in females during proestrus, when circulating estrogens are at their highest, analgesic responsiveness to intrathecal EM2 is indistinguishable from males and are not influenced by blockers of either ERα, ERβ or GPR30.[34] However, during diestrus, when peripheral estrogens are relatively low, blockade of spinal mERα or GPR30 restores spinal EM2 analgesia to that manifest in untreated proestrous female or male rats.[34] Thus, relatively low levels of circulating estrogens in females are (temporally) associated with maximum estrogenic suppression of spinal EM2 analgesia, the neutralization of which could have substantial translational value in pain control. Additionally, this underscores the dichotomous relationship between spinal and circulating estrogens.

CNS synthesis of estrogens:

The spinal and supraspinal CNS contain a wide distribution of the enzyme aromatase, which enables CNS in situ synthesis of estrogens. Moreover, aromatase is present in many spinal areas involved in nociception/opioid antinociception.[36–40] Estrogens intrinsic to the spinal cord are in fact essential to the suppression of intrathecal EM2 analgesia, as evidenced by the ability of the intrathecally applied aromatase inhibitor fadrozole to uncover spinal EM2 analgesia similar in robustness to the EM2 analgesia observed in proestrous female and male rats.[34]

Estrogens and ERs partner with mGluR$_1$:

In addition to spinal mERs (mERα, GPR30) and aromatase, mGluR$_1$ is also required for diestrous-associated suppression of spinal EM2 analgesia, but even this effect is inextricably linked to estrogenic signaling. During diestrus, the noncompetitive mGluR$_1$ antagonist YM298198 rapidly (within 5 min) uncovers a robust spinal analgesic response to intrathecal EM2 that is neither distinguishable from that manifest in proestrus female and male rats[34] nor from the response elicited by intrathecal EM2 during diestrus following mERα blockade or aromatase inhibition. The parallelism between effects of blocking mGluR$_1$ and blockade of mERα/aromatase inhibition led to our hypothesizing that mERα acted to modify mGluR$_1$ signaling by physically interacting with it, as has been described for other signaling proteins.[41,42] In this scenario, the presence of mERα in mGluR$_1$ spinal cord immunoprecipitate is significantly greater in immunoprecipitate obtained during diestrus than proestrus, consistent with mGluR$_1$ collaborating with estrogenic-mERα signaling to suppress intrathecal EM2 analgesia during diestrus but not proestrus.

Parallelism between pharmacological and intrinsic regulation of spinal EM2 antinociception:

Notably, MOR, aromatase, mERα, and mGluR$_1$ not only have wide distribution in the dorsal horn, but also are coexpressed and colocalize in or near the plasma membrane of neurons during diestrus.[34] Furthermore, EM2-immunoreactive varicosities appose the dendrite of a MOR-immunoreactive neuron (in the lumbar region of the spinal cord) coexpressing mGluR$_1$ and mERα. This provides a cellular context for spinal mERα, mGluR$_1$ and estrogens not only to be synthesized within the same neuron, but also to negatively modulate intrathecal EM2 analgesic responsiveness during diestrus. Thus, estrous cycle stage-correlated targets are emerging (eg, mERs, mGluR$_1$ aromatase) for magnifying opioid

analgesic responsiveness to spinally applied EM2, and perhaps endogenously generated EM2 that have substantial potential translational value.

Stage of cycle-correlated emergence of signaling sequelae that facilitate spinal EM2 analgesia:

In addition to signaling molecules that are active in suppressing intrathecal EM2 analgesia, there are also signaling molecules that sustain spinal EM2 analgesia during proestrus; the emergence of robust intrathecal EM2 analgesia during proestrus does not result solely from the loss of aromatase-mERα-mGluR$_1$ suppression (prominent in diestrus) of intrathecal EM2 analgesia, but also requires the emergence of alternative facilitative signaling. These include a switch from mERα to glutamate activation of mGluR$_1$, and a critical requirement for threshold levels of spinal dynorphin release and KOR activation.[43]

Reciprocal relationship between intrathecal EM2 analgesic responsiveness and spinal.

Release of endogenous EM2: Release of endogenous spinal EM2 is itself also tightly controlled over the rat estrous cycle. Notably, however, negative regulation of spinal EM2 release is mediated by estrogens and mERs. Additionally, in contrast to intrathecal EM2 analgesic responsiveness, it is robust during proestrus and much less so during diestrus (and absent during estrus.[44] This parallels the highs and lows of peripheral estrogens, highest in proestrus (146.8–367 pM), lower in diestrus (up to 135.8 pM), reaching the nadir in estrus (down to 18.40 pM). Furthermore, underscoring the intricacy and complexity of this regulation, not only is the concomitant action of both mERα and GPR30 a prerequisite for suppression of i.t. EM2 analgesia,[44] but both peripherally as well as spinally synthesized estrogens are required. This is evidenced by the ability of either ovariectomy or spinal aromatase inhibition to eliminate proestrous-associated estrogenic suppression of spinal EM2 release.[44] The ability of estrogens to function as a biological lock on EM2 release is underscored by the inverse association between basal EM2 release and peripheral levels of estrogens. The mechanism(s) mediating combinatorial interactions between centrally and peripherally synthesized estrogens are not currently understood.

Endogenous biased agonism: The difference in spinal EM2 analgesic responsiveness over the estrous/menstrual cycle is contingent on whether or not mGluR$_1$ is activated by glutamate or mERα, as well as the ebb and flow in spinal dynorphin/KOR signaling. Suppressive vs. facilitative variation of spinal EM2 analgesia by mGluR$_1$ signaling that depends on the endogenous activator of mGluR$_1$ most likely reflects endogenous biased agonism—agonist-induced conformations of receptor that preferentially stimulate certain signaling pathways over others. This is potentially particularly relevant to signaling by mGluR$_1$, since mGluR$_1$ functionally associates with G$_q$,[45] as well as G$_s$[46,47] and G$_{i/o}$,[48,49] the degree of this association being influenced by mERα vs. glutamate activation of mGluR$_1$. Our present finding that spinal EM2 analgesia is both inhibited and facilitated by spinal mGluR$_1$, depending on its activator, strongly suggests that ligand bias is not only relevant to exogenously applied agonists, as a pharmacological construct, but is, additionally, also likely to be an endogenous controlling mechanism. Interestingly, EM2 itself is reported to be a biased agonist at MOR.[50,51]

Endogenous spinal estrogenic signaling does not alter intrathecal EM2 analgesia during proestrus,[43] indicating the disengagement of estrogens from causally associated underlying processes. However, paradoxically, acute spinal $mGluR_1$ blockade (via intrathecal YM298198) (that reveals intrathecal EM2 analgesia during diestrus) actually abolishes spinal EM2 analgesia during proestrus. This indicates that the conversion from spinal EM2 non-analgesic to analgesic responsivity during diestrus and proestrus, respectively, results from the emergence of $mGluR_1$ facilitative effects during proestrus that was not present during diestrus, in addition to the negation of suppressive mERα-$mGluR_1$ modulation.

Relevance of the ebb and flow of spinal dynorphin/KOR signaling to intrathecal EM2 analgesia: The ability of intrathecal EM2 to produce analgesia is determined by variability in spinal dynorphin release, repressed in diestrus but facilitated in proestrus. During proestrus, dynorphin release into spinal perfusate is augmented nearly 2-fold relative to that achieved during diestrus.[43] This is consistent with our earlier pharmacological demonstration that spinal dynorphin/KOR activity is essential for female, but not male, intrathecal EM2 analgesic responsiveness.[7] In fact, spinal dynorphin/KOR activity is a prerequisite for the ability of mERα/$mGluR_1$ blockade to uncover intrathecal EM2 analgesic responsiveness during diestrus.[43] Either intrathecal anti-dynorphin antibodies (30 min prior to EM2) or intrathecal norbinaltorphimine (norBNI; KOR-selective antagonist; 18 h prior to EM2 intrathecal treatments) abolished the intrathecal EM2 analgesia that emerged after blocking either spinal mERα or $mGluR_1$.[43] These data suggest that during diestrus, unmasking spinal EM2 analgesia by either spinal mERα or $mGluR_1$ blockade results from disinhibiting spinal dynorphin release and KOR signaling, as well as facilitating signaling by glutamate/$mGluR_1$, implying the prerequisite for threshold levels of their endogenous signaling activities for intrathecal EM2 analgesia to be manifest. This formulation is buttressed by the ability of intrathecal EM2 to produce analgesia during diestrus when rats are intrathecally pretreated (30 min prior to intrathecal EM2) with intrathecal dynorphin itself (3 nmol)[35,43] and the facts that acute blockade of spinal glutamate release with intrathecal pretreatment with riluzole (glutamate release inhibitor[52,53]; 43 nmol, 1 hr) eliminated intrathecal proestrous-associated intrathecal EM2 analgesia, reducing it to levels observed in diestrus females,[43] while blockade of glutamate transport (reuptake) (which enhances synaptic glutamate) unmasks spinal EM2 analgesia during diestrus (Liu and Gintzler, unpublished observations).

Anatomical organization of spinal EM2, $mGluR_1$, ERα, glutamate and dynorphin; exogenous vs. intrinsic regulation: Confocal imaging reveals that a thin shell of dynorphin-immunoreactivity envelops neurons, as we have previously described.[54] Moreover, such neurons coexpress $mGluR_1$ and ERα in or adjacent to the plasma membrane, as well as within the cell body. Importantly, these glutamate transport vesicles (VGLUT)-expressing (glutamatergic) terminals appose these neurons, affording a cellular basis for cycling among ERα-activated and glutamate-activated $mGluR_1$ signaling over the estrous cycle. This organization permits modulation of spinal dynorphin release by glutamate, thereby coordinating glutamate activation of $mGluR_1$ with dynorphin release, both of which are essential for spinal EM2 analgesia during proestrus.[43]

Do endogenous opioids mediate endogenous antinociception?—An abundance of evidence indicates that endogenous opioids (β-endorphins,[55,56] endomorphins,[57] dynorphins,[58] and enkephalins)[59] mediate placebo-induced analgesia.[60–64] Moreover, they do so via the same neural mechanisms that mediate opioid analgesia produced by narcotics.[65] This is underscored by reports that the magnitude of analgesia produced by synthetic opioids directly correlates with the magnitude of placebo-induced opioid analgesia.[65] This portends that endogenous and exogenous opioids share common mechanisms and, furthermore, that harnessing endogenous opioid analgesia for chronic pain management is likely to have translational utility. Amazingly, spatially-directed expectation of pain relief not only produces endogenous opioid-mediated pain reduction,[66] but does so only on the body part targeted by the expectation.[67]

Additional evidence that endogenous opioids are active as analgesics in situ include the following: (1) placebo-induced elimination of postoperative dental pain is abolished by opioid receptor block,[60] which also augments clinical nociception[61]; (2) anticipation of pain relief itself activates human MOR[62]; (3) analgesia triggered by a placebo occurs concomitant with amplified endogenous opioid action[68]; (4) transcranial direct current stimulation-induced analgesia enhances MOR recruitment[69]; (5) tissue injury constitutively activates MOR, suppressing spinal nociception[63]; (6) endogenous opioid activity is elicited by transcranial magnetic stimulation resulting in opioid analgesia[64]; (7) opioid antagonists block analgesic effects of acupuncture and electroacupuncture.[70–72] Moreover, responsiveness to opioid analgesics can predict the magnitude of opioid placebo responsiveness, underscoring shared opioid mechanisms and the likely clinical utility of eliciting endogenous analgesia for chronic pain management. These instances validate that harnessing endogenous opioids can be an effective analgesic strategy. However, they do not provide pharmacological targets for effectively doing so under commonly encountered clinical situations, nor the likely success in doing so.

Endogenous opioids and clinical pain control.—A critical question is whether or not endogenous opioid analgesia can be subject to pharmacological activation, within the time frame required for the opioid analgesia elicited by exogenous synthetic narcotics. The unleashing of intrathecal EM2 analgesia during diestrus by either (1) inhibiting spinal aromatase or (2) blocking spinal mERα, or (3) blocking spinal mGluR$_1$, combined with the active facilitation of intrathecal EM2 analgesia during proestrus by spinal glutamate and dynorphin release/KOR activation,[34,43] provides a roadmap for pharmacologically turning on CNS endogenous opioid analgesia (particularly that resulting from EM2), and assessing the potential clinical utility of doing so (Fig 1). Both are required if tapping into endogenous opioid analgesia is to fulfill its promise of a viable clinical alternative to synthetic prescription opioids, enabling access to the powerful analgesic properties of opioids while minimizing their abuse potential.

Understanding the on/off switch of intrathecal EM2 analgesic mechanisms could point the way for developing pharmacotherapies for manipulating endogenous EM2 activity for medicinal purposes. Plastic interactions within a membrane-bound oligomer that contains ERs, aromatase, mGluR$_1$, mGluR$_{2/3}$, MOR and KOR[43] underlies estrous cycle-associated phasic changes in analgesic responsiveness to spinal EM2. As discussed above and shown in

Fig 1, spinal cord contains an anatomical organization that permits endogenous interactions among modulatory components of EM2 analgesia analogous to those pharmacological treatments that 'turn on and off' analgesia elicited by the exogenous (intrathecally) applied EM2. Thus, pharmacologic perturbations that unveil analgesic responses to intrathecal EM2 during diestrus (eg, mERα/mGluR$_1$ blockade, aromatase inhibition, inhibition of glutamate transport) would be expected to enhance endogenous spinal opioid analgesia. In analogous fashion, pharmacologic perturbations that sustain analgesic responses to intrathecal EM2 during proestrus (eg, glutamate activation of mGluR$_1$, dynorphin release), would also be expected to undergird endogenous opioid analgesia, both sets of pharmacological perturbations producing effects in an estrous cycle-correlated fashion. Alternatively, whereas spinal mGluR$_1$ blockade in diestrus would be expected to be analgesic/antiallodynic, the same treatment during proestrus would be expected to produce the opposite, be pronociceptive, ie, exacerbate nociception. Accordingly, intrathecal EM2 modulatory dynamics defined thus far establishes guardrails for translational forays into establishing the 'reasonableness' of pharmacologically tapping into intrinsic opioid systems for clinical pain relief in women.

Proof of principle that intrinsic opioid analgesic systems can be pharmacologically activated within a requisite time frame for clinical utility: During physiologically quiescent conditions (ie, in the absence of nociception), endogenous opioid systems are dormant; opioid receptor block fails to alter basal nociceptive thresholds in laboratory animals[73,74] and humans.[75,76] However, nociceptive stimuli do produce endogenous opioid analgesia,[60,77–80] indicating the ability of those stimuli to release endogenous opioids. This suggests the utility of using a chronic pain model to investigate whether or not pharmacological interventions that enhance analgesic responsiveness to exogenous, intrathecal EM2, also augment endogenous opioid analgesia. Accordingly, we utilized spinal nerve ligated diestrous rats, a known pain model to establish that opioid-mediated anti-allodynia could be provoked in the absence of exogenous opioids via the same pharmacological treatments that unveil spinal analgesic responsiveness to intrathecal EM2 in intact diestrous rats. Spinal nerve ligation (SpNL) was selected as the chronic pain model,[81–83] since it augments release of endogenous opioids. This is reflected by the ability of spinal opioid receptor blockade to exacerbate mechanical allodynia,[84] which is not observed in surgically naïve rats (Liu and Gintzler, Unpublished observations).

As we had predicted, the mechanical allodynia manifest by SpNL in diestrous rats, is markedly attenuated by either spinal aromatase inhibition or mERα/mGluR$_1$ blockade (Table I). This is manifest only on the paw ipsilateral to SpNL and, importly, is eliminated by naloxone. The latter indicates endogenous opioid mediation, notwithstanding that no exogenous opioid had been administered. In other words, the opioid anti-allodynia resulted from harnessing the activity of an intrinsic opioid(s).

Asynchronous relationship between circulating estrogens and CNS estrogen functionalities: Estrogenic signaling in the spinal cord is a crucial parameter influencing spinal EM2 analgesia. In diestrus, rapid signaling spinal mERs, activated by spinally synthesized estrogens, suppress spinal EM2/MOR analgesia, consistent with the presence of

aromatase in many spinal areas involved in nociception and opioid analgesia.[36,37,39] Surprisingly, the adverse effect of spinally synthesized estrogens on intrathecal EM2 analgesia occurs during diestrus,[34] when circulating levels of estrogens are relatively low, not during proestrus, when systemic levels of estrogens are at their highest. This enigmatic relationship between spinal and peripheral estrogens indicates that cycle stage does not always dependably forecast the magnitude of estrogenic signaling in the CNS, informing attempts to modulate CNS estrogenic signaling for medicinal purposes. Furthermore, this inverse relationship is likely a basis for many inconsistent findings regarding nociception and opioid antinociception over the estrous and menstrual cycle,[85] often a major confound in investigating the male-female dichotomy in pain, as well as pain management in women.

Existing data supports the existence of at least 2 estrogenic systems, one in the CNS and one, ovarian-based, in the periphery. The interrelationships between these estrogenic systems are mostly unexplored but have been the subject of much speculation.[86] Peripheral estrogens reach the CNS by penetrating the blood brain barrier and diffusing from cerebrospinal fluid to extracellular fluid[87] and sites of action. Such estrogens likely act directly on spinal ERs and might be expected to produce a generalized stimulation of CNS mERs, trivializing the functionality of estrogens synthesized in the CNS. However, systemic estrogens do not have unhampered access to all CNS ERs, the accessibility being influenced by the activity/distribution of estrogen-metabolizing enzymes,[88–93] estrogen binding proteins, etc. In keeping with restricted access of systemic estrogens to CNS sites of action, some signaling by estrogens in the CNS is strikingly out-of-phase with peripheral concentrations of estrogens,[34] which would not be expected if systemic estrogens had unrestricted access to CNS ERs.

In parallel with systemic estrogens, CNS estrogens are synthesized by spinal aromatase near synaptic structures and also stimulate proximal ERs.[36,37,39,40] Spinal cord estrogens act in-phase as well as out-of-phase with peripheral estrogens. Since diffusion of centrally synthesized estrogens is highly spatially restricted,[86,94–96] it is not improbable that CNS-and ovarian-derived estrogens not only activate different populations of spinal ERs that are either functionally convergent or parallel and independent, but also have variable temporality. Furthermore, the proclivity of membrane aromatase to physically pair with or exist separately from membrane estrogen receptor a[97] creates 2 populations of aromatase, each of which themselves can be in-phase and/or out-of-phase with the ebb and flow of estrogens across the estrous cycle.

Our studies compare two well-characterized endocrinological states, diestrus and proestrous (the longest estrous cycle stages facilitating behavioral analyses) in order to generate two relative homogeneous populations of animals as well as to have markers of estrogenic activity in the periphery. However, direct causality between stage of cycle and endogenous spinal EM2 functionality is not, necessarily to be inferred. Consequently, given the often dichotomous relationship between central and peripheral estrogenic activity, further refinement correlating spinal EM2 analgesic functionality with additional estrous cycle stages (eg, metestrus, estrus) have not been pursued. Similarly, in this review, we do not provide a comparison between stage of estrous and menstrual cycle circulating estrogen levels since points of intersection between central and peripheral estrogens across rodents

and primates are not understood and thus stage of cycle comparisons could prove very misleading regarding CNS analgesic functionality.

In rat, continuing the parallel between the ebb and flow of intrathecal EM2 analgesic responsiveness and stage of cycle, in proestrus, $mGluR_1$ antagonism, which abolishes intrathecal EM2 analgesia in untreated proestrous rats, markedly aggravated the SpNL-induced mechanical allodynia of the ipsilateral paw in proestrous rats (Table I).[84] Temporal correlation of pain management outcome on stage of reproductive cycle has substantial translational consequences, mandating that stage of menstrual cycle be tracked and considered when employing pharmacotherapies to tap into endogenous opioid analgesia in women of childbearing age. This is particularly important since pharmacotherapies that are antinociceptive in one stage can be pronociceptive in another.

Oligomerization of aromatase and mERα: As part of investigating mechanisms responsible for the ups and downs of intrathecal EM2 analgesia, we discovered that aromatase and mERα oligomerize to form signaling complexes.[34] This enables a novel modality of estrogenic signaling that we termed ‘oligocrine’, the ability of estrogens to perform as intracellular messengers, which are synthesized and act within the same macromolecular signaling complex.[34] From a translational perspective, it can be important to note that these complexes can be independently regulated,[34] constituting a molecular structure mediating the differential influence of stage of reproductive cycle on nociception and opioid analgesia.

As an exemplar, variable connection of cycle stage with the activity of discrete subpopulations of estrogenic signaling complexes[34] could underlie inconstant nociception/opioid antinociception across the reproductive cycle.[1–7] Potential translational relevance of discrete subpopulations of mERα-aromatase signaling complexes is bolstered by the fact that the extent of the oligomerization between aromatase and mERα vary in spinal cord (that has predominantly neural functionalities) and hypothalamus that has both endocrine and neural functionalities. Specifically, in spinal cord, regardless of reproductive cycle stage, virtually all membrane aromatase is oligomerized with mERα.[97] In contrast, only ≈15% is oligomerized in hypothalamus.[97] Furthermore, the prevalence of non-mERα-associated membrane aromatase in hypothalamus, in combination with the fact that numerous hypothalamic aromatase immunoreactive neurons are retrogradely labeled with peripherally injected Fluoro-Gold[97] (ie, extend outside of the blood brain barrier), implies that some estrogens are secreted from the hypothalamus, possibly to regulate pituitary function, which could be exploited for medicinal purposes. The occurrence of membrane aromatase and mERα associated and non-associated subpopulations in the CNS holds out the promise that their selective targeting restores impaired estrogen-dependent CNS functionalities while curtailing undesirable effects.

Oligocrine estrogenic signaling also provides a structural context for fluid relationships of cycle stage-associated peripheral estrogens with estrogenic functionalities. For example, in proestrus, spinal aromatase and mERα activity are both essential for MOR-KOR heterodimerization,[98] the analgesic form of KOR.[99] In stark contrast, whereas spinal aromatase/mERα activity *does not* modulate spinal EM2 analgesia in proestrus, it does

during diestrus.[34] These variances infer that separate populations of locally synthesized estrogens independently activate discrete pools of spinal mERα. This realization underscores the imperative to be cautious when associating particular CNS functionalities with circulating ovarian steroid levels, and to consider cycle stage as a covariate in females of childbearing age in all investigations of nociception, as well as opioid analgesia and translational forays therein.

In many ways, the opioid field developed backwards. We extensively characterized narcotic drugs and the pharmacology of the receptors it was theorized they acted upon before we were able to appreciate the endogenous ligands whose effects they were mimicking. As a result, salient functional characteristics of narcotic drugs were thought to apply, often inappropriately, to endogenous opioids. We now know that this was overly simplistic. A poignant example of such differences is the characteristics of the spinal analgesia produced by EM2[100-103] versus the intrathecal opioid analgesia resulting from the decidedly selective MOR-selective agonists sufentanil or [D-Ala2,N-Me-Phe4,Gly-ol5]-enkephalin (DAMGO). Intrathecal application of either sufentanil or DAMGO produces strong analgesia in both female (irrespective of the stage of estrous cycle) and male rats that is naloxone-reversible and does not oscillate between analgesically active and inactive states,[7] as does intrathecal EM2. Additionally, mechanisms underlying sufentanil or DAMGO spinal opioid analgesia vary from those utilized by EM2, notwithstanding that all 3 MOR ligands are highly selective for their targeted receptor. Sufentanil and DAMGO act entirely via MOR to produce analgesia, irrespective of sex or stage of cycle, whereas EM2 requires spinal dynorphin and KOR signaling, concomitant with MOR activation, to produce analgesia in proestrous female rats.[7] These variances are consistent with the fact that spatiotemporal activation patterns of opioid receptors are differentially produced by native opioid peptides and narcotics drugs.[104] Such observations profile the translational relevance of extensive characterization of exogenous responsiveness to endogenous opioids and the neuronal circuits that mediate them when attempting to develop pharmacodynamic therapies designed to harness intrinsic opioid analgesic systems for acute and chronic pain management.

Pain management in the era of the epidemic of prescription opioid abuse: Treating chronic pain is confounded by concerns regarding addiction to and misuse of opioid analgesics. The persisting quandary is how to continue benefiting from the unparalleled pain-mitigating properties of opioids while Eradicating addiction to and misapplication of opioids, ie, balancing the social command to equalize the urgency to allay the unrelenting opioid misuse epidemic with the ethical necessity to heal and effectively manage pain. The pharmacological harnessing of endogenous opioids for targeted pain relief may hold promise for pain management by allowing utilization of opioids while avoiding confounds of addiction and prescription opioid misuse.

Undermanaging pain has substantial deleterious functional consequences: The present environment encourages the implementation and enforcement of policies that rigidly restrain the medical employment of synthetic opioids for pain management. As well intentioned as that might be, it is critical to understand that inadequately managed pain is itself a health risk. Uncontrolled or poorly managed pain has

been reported to alter brain structure and function, modifying the functional connectivity of cortical regions[105] and decreasing gray matter in pain-transmitting areas,[106] prefrontal cortex and thalamus.[107] In addition to these physical deleterious consequences, chronic pain has been associated with poor sleep, depression and anxiety,[108] impaired emotional decision-making[109] and diminished motivation (via long-term depression in the nucleus acumbens).[110]

CONCLUSION

The catalogue of negative effects of poorly managed pain emphasizes that just saying no to the medicinal use of synthetic opioids for pain management is not an acceptable way to get a handle on the opioid misuse crisis that is plundering society. A vital problem demanding consideration is how to resolve the medical and ethical necessities to assuage chronic pain with the ongoing rampant opioid crisis. Endogenous opioids represent an untapped reservoir of less easily abused opioids. Utilization of the newfound complexities of CNS estrogenic functionalities and the ability of glutamate/mGluR$_1$ and dynorphin/KOR to sustain endogenous opioid analgesia (during proestrus) could bring drugs that act to harness endogenous opioids into the mainstream pharmacopeia, expanding effective pain management options.

The biological underpinnings for the strikingly severity and frequency of chronic pain in women than men remain an enigma. Notably, while the flexibility of EM2 utilization may contribute to women's elevated risk of developing chronic pain conditions, it also provides an opportunity for medicinal intervention. CNS mechanisms underlying any cooperative/synergistic actions of central and peripheral estrogens could represent novel pharmacological targets for ameliorating pain in women, facilitating enhanced utilization of endogenous EM2. Given the variable in-phase and out-of-phase nature of estrogenic functionalities with stage of cycle, it appears that generalizations across estrous and menstrual cycling humans would have to be empirically determined.

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Abbreviation:

Aro	aromatase
CNS	central nervous system
DAMGO	D-Ala2,N-Me-Phe4, Gly-ol5]-enkephalin
E2	17-β-estradiol
EM2	endomorphin 2
ERβ	estrogen receptor beta
ERs	estrogen receptors

Glut	glutamate
hr	hour
KOR	kappa-opioid receptors
mERα	membrane estrogen receptor alpha
mERs	membrane estrogen receptors
mGluRs	metabotropic glutamate receptors
mGluR$_1$	metabotropic glutamate receptor 1
MOR	μ-opioid receptor
norBNI	norbinaltorphimine
SpNL	spinal nerve ligation
VGLUT	vesicular glutamate transport vesicles

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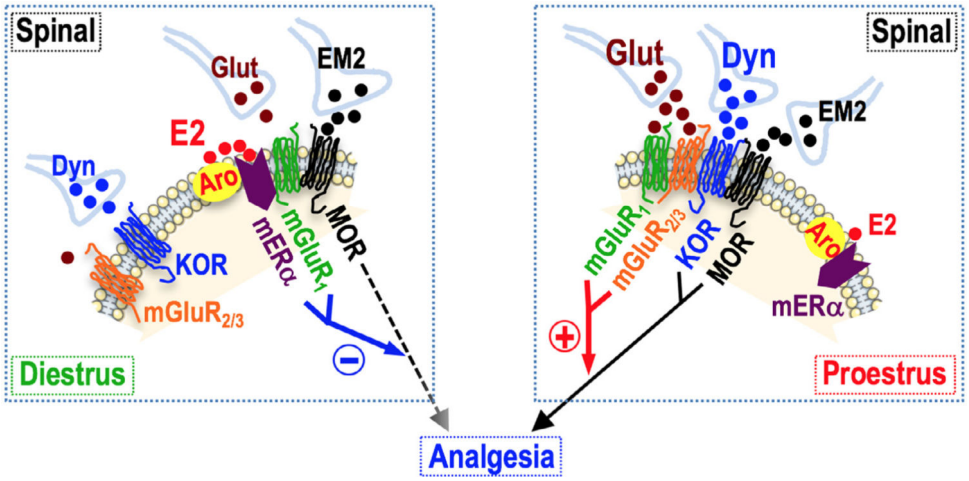
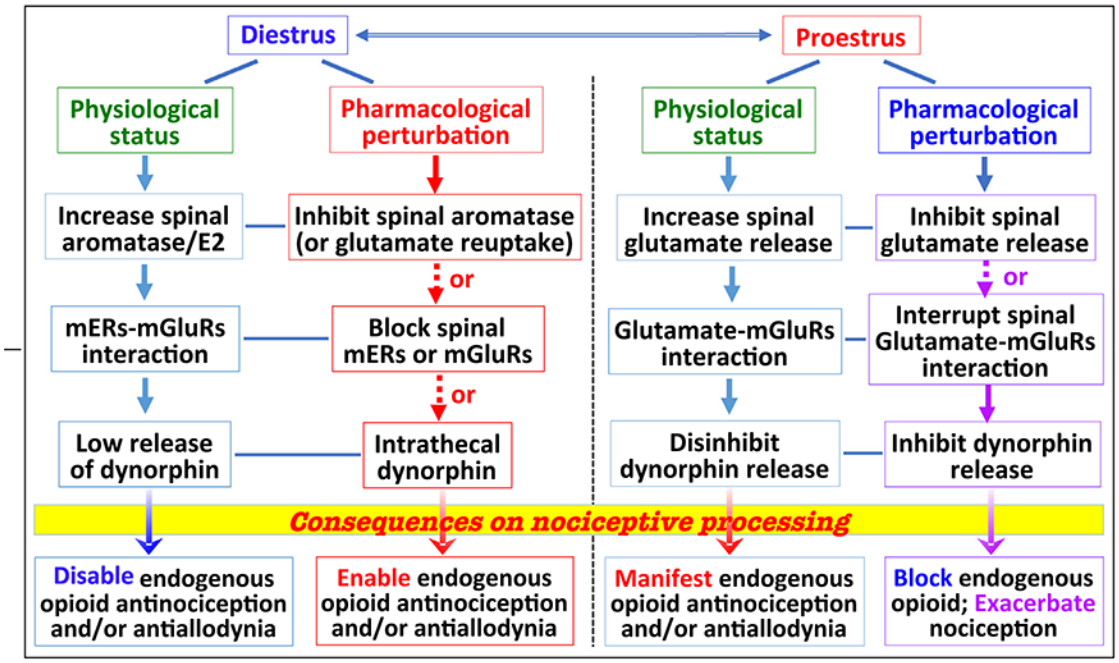


Fig 1.

Analgesic responsiveness to spinal EM2 is governed by dynamic, pliable interactions among MOR, KOR, $mGluR_1$, $mGluR_{2/3}$, mERα and aromatase (Aro) within an oligomer that tracks the estrous cycle. In diestrus, E2 synthesized within the oligomer comprised of Aro-mERα-$mGluR_1$-MOR stimulates $mGluR_1$ via signaling by mERα to suppress analgesic responsiveness to intrathecal EM2 by inhibiting MOR signaling. Blockade of mERα/$mGluR_1$ or inhibition of Aro neutralizes MOR inhibition, unmasking endogenous MOR-mediated (EM2) analgesia. The disconnection of suppressive mERα-$mGluR_1$ signaling, the transition from mERα to glutamate (Glut) activation of $mGluR_1$, which now signals in partnership with $mGluR_{2/3}$, and augmented spinal Dyn/KOR signaling, which signals in collaboration with MOR in an oligomer of $mGluR_1$-$mGluR_{2/3}$-KOR-MOR that is different from that of diestrus, triggers the appearance of spinal EM2 analgesia. Inhibition of Glut release and thus a decrease in $mGluR_1$/$mGluR_{2/3}$ signaling activity eliminates the expression of endogenous spinal opioid analgesia, resulting in the worsening of allodynia in neuropathic pain rats. An organizational framework in which the spinal neurons coexpressing the pertinent signaling proteins (oligomerized therein) are in apposition to EM2-expressing, Dyn/Glut-containing varicosities likely underlies these observations. This organization would permit individual neurons to vary responsiveness to EM2 as a function of the ebb and flow of spinal Dyn and Glut signaling.

Table I.

Endogenous opioid blunting of nociceptive processing is regulated by dynamic interactions among signaling molecules



In diestrous female rats, interactions between mERs activated by spinally synthesized estrogen and $mGluR_1$, combined with attenuated release of spinal dynorphin, actively suppress intrathecal EM2 analgesia, as well as the ability of endogenous spinal opioids to dampen the allodynia resulting from SpNL. This suppression can be overcome by enhancing spinal synaptic glutamate and/or spinal $mGluR_1$ and KOR activity. The robust endogenous opioid dampening of SpNL-induced allodynia during proestrus requires spinal glutamate (in lieu of estrogen) activation of spinal mGluRs, as well as a threshold activation of spinal KOR by spinal dynorphin, whose release is augmented during proestrus. Linkage of endogenous opioid analgesia/antiallodynia to physiological state, and deciphering responsible molecular underpinning(s), holds out promise of defining pharmacological interventions to harness endogenous opioids for medicinal pain relief, while minimizing opioid abuse potential.