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Exploiting endogenous opioids: Lessons learned from endomorphin 2 in the female rat

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Abstract

Effective management of chronic pain is demanded by ethical as well as medical considerations. Although opioid analgesics remain among the most effective pharmacotherapies for ameliorating many types of pain, their use is clouded by concerns regarding their addictive properties, underscored by the current epidemic of prescription opioid abuse and attendant deaths. Medicinal harnessing of endogenous opioid antinociception could provide a strategy for continuing to take advantage of the powerful antinociceptive properties of opioids while avoiding their abuse potential. Based on our studies of endogenous mechanism that suppress and facilitate spinal endomorphin 2 antinociception over the rat reproductive cycle, we identified multiple signaling molecules that could serve as targets for activating endogenous opioid analgesia for chronic pain management in women. Our findings emphasize the need for a precision medicine approach that includes stage of menstrual cycle as an important determinant of drug targets for (activating/harnessing) endogenous opioid antinociceptive systems/capabilities. Utilization of drugs that harness endogenous opioid antinociception in accordance with varying physiological states represents a novel approach for effective pain management.

Keywords

Endogenous opioids; Endomorphin 2; Estrogenic signaling; Spinal cord; Reproductive cycle; Antinociception; Dynorphin

1. Introduction

The current prescription opioid abuse epidemic underscores the need to develop new approaches to harnessing the powerful antinociceptive properties of opioids while avoiding their abuse potential. This focuses renewed attention on formulating strategies for tapping into the endogenous opioid system for pain relief. Doing so requires an understanding of how endogenous opioid activity is regulated and identifying components of that regulation that could serve as novel drug targets.

Disclosures

None of the authors hold any conflicts of interest.

1.1. Antinociception via endogenous opioids

Multiple observations have established that activation of endogenous opioid systems can produce antinociception over a spectrum of commonly experienced nociceptive insults: (a) opioid receptor blockade eliminates placebo-induced reduction of postoperative dental pain [1] and enhances clinical nociception [2]; (b) expectation of pain relief activates mu-opioid receptors (MOR) in human brain [3]; (c) placebo analgesia correlates with increased endogenous opioid activity [4]; (d) analgesic effects reported with transcranial direct current stimulation results in part, from enhanced recruitment of MOR [5]; (e) tissue injury leads to constitutive activation of MOR that can suppress spinal nociception [6]; (f) repetitive transcranial magnetic stimulation produces acute and chronic analgesic effects that derive from endogenous opioidergic activity [7]; (g) analgesic effects of acupuncture and electroacupuncture are blocked by naloxone, indicating mediation by endogenous opioids [[8], [9], [10]]. These examples demonstrate that harnessing endogenous opioids can be an effective antinociceptive strategy. However, they do not provide pharmacological targets for effectively doing so under ordinary clinical situations.

1.2. Functional aspects of endogenous vs. clinically used opioids

Characteristics of the action of exogenously applied opioids have heavily influenced perspectives on endogenous opioid functionality. However, there are some important differences between the mechanisms underlying the analgesic effects of some endogenous opioid peptides and the opioids commonly used clinically. These differences are notable because they provide insights into how endogenous opioid activity might be manipulated for therapeutic purposes, thereby circumventing the potential for prescription opioid abuse.

An exemplar of such differences is the characteristics of the spinal antinociception produced by the endogenous MOR ligand endomorphin 2 (EM2) [[11], [12], [13], [14]] vs. the spinal antinociception produced by the highly selective MOR agonists sufentanil or [D-Ala²,N-Me-Phe⁴,Gly-ol⁵]-enkephalin (DAMGO). Intrathecal (i.t.) sufentanil or DAMGO produce potent naloxone-reversible antinociception in both female (irrespective of the stage of estrous cycle) and male rats. In contrast, the antinociception resulting from i.t. EM2 oscillates between analgesically active and inactive states [15]: during diestrus (when circulating levels of estrogens are low) spinal EM2 antinociceptive responsiveness is essentially undetectable, whereas during proestrus (when circulating estrogens are high), spinal EM2 antinociception is robust and comparable in magnitude to that manifest by males.

1.3. Differential mechanisms used by EM2 vs. synthetic MOR agonists

Mechanisms underlying sufentanil or DAMGO spinal antinociception also differ from those used by EM2, despite the fact that all three agonists are highly selective for MOR. Sufentanil and DAMGO act exclusively via MOR to produce antinociception. In contrast, EM2 requires spinal dynorphin (Dyn) and kappa-opioid receptor (KOR) signaling, concomitant with MOR activation, to produce antinociception [15]. Strikingly, the antinociception produced during proestrus by i.t. EM2 mechanistically diverges from the analogous antinociception produced during diestrus by i.t. morphine [16]. (Although morphine is less MOR-selective than EM2, DAMGO or sufentanil, it is often classified as a MOR agonist.)

These mechanistic differences (summarized in Table 1) are consistent with a recent report indicating that native opioid peptides and opioid drugs differentially elicit spatiotemporal activation patterns of opioid receptors [17].

The divergence of antinociceptive mechanisms used by EM2 and sufentanil/DAMGO is consistent with the ability of EMs, but not sufentanil or DAMGO, to act as β -arrestin-biased ligands, preferentially activating β -arrestin-dependent signaling [18,19]. This observation by itself suggests novel targets for coopting components of endogenous opioid antinociceptive mechanisms in lieu of exogenous opioid analgesics. Furthermore, and perhaps of even greater potential clinical importance, the absence of spinal EM2 antinociception during defined stages of the estrous (and presumably menstrual) cycle and impaired transition from spinal EM2 analgesically nonresponsive to responsive physiological states could underlie the abundant evidence that women experience greater severity and frequency of chronic intractable pain syndromes than do men [20,21].

Understanding the on/off switch of spinal EM2 analgesic mechanisms could point the way for developing pharmacotherapies for manipulating endogenous EM2 activity for medicinal purposes. To this end, we now know that phasic changes in analgesic responsiveness to spinal EM2 result from plastic interactions within a membrane-bound oligomer that contains estrogen receptors, aromatase (aka estrogen synthase), metabotropic glutamate receptor 1 (mGluR₁), metabotropic glutamate receptor 2/3 (mGluR_{2/3}), MOR and KOR [22].

1.4. Physiological modulation of spinal EM2 antinociception over the estrous cycle

CNS estrogenic signaling is a critical determinant of spinal EM2 antinociception. During diestrus, rapid signaling spinal membrane estrogen receptors (mERs), activated by spinally synthesized estrogens, suppress spinal EM2/MOR antinociception. This suppressive role of locally synthesized estrogens is consistent with the presence of aromatase in many spinal areas involved in nociception and opioid antinociception [[23], [24], [25]]. Notably, the negative influence of spinally synthesized estrogens on i.t. EM2 analgesia occurs during diestrus [26], when circulating levels of estrogens are low, not in proestrus, during which systemic levels of estrogens peak. This paradoxical relationship between spinal and systemic estrogens indicates that cycle stage does not reliably predict the magnitude of estrogenic signaling in the CNS.

Moreover, this inverse relationship likely underlies inconsistent findings regarding the magnitude of nociception and opioid antinociception over the estrous and menstrual cycle [27], often a major confound in investigations of the male-female dichotomy in pain.

1.5. Membrane complexes containing aromatase and ER α permit estrogenic signaling to be inversely related to circulating estrogens

As mentioned above, EM2 antinociception in females varies over the estrous cycle. Recently, we demonstrated that aromatase and mER α form signaling complexes [26], enabling a new modality of estrogenic signaling. We termed this type of signaling ‘oligocrine’-- the ability of estrogens to function as intracellular messengers that are synthesized and act within the same macromolecular signaling complex [26]. Importantly, these complexes can be independently regulated [26], providing a molecular organization for

the influence of stage of estrous cycle on nociception and opioid antinociception. For example, differential association of stage of estrous cycle with spinal estrogenic signaling within discrete subpopulations of signaling complexes [26] could account for variable nociception/opioid antinociception across the reproductive cycle [15,20,21,[28], [29], [30], [31]].

Oligocrine estrogenic signaling also provides an organizational context for variable association of cycle stage (circulating estrogens) with estrogenic functionalities. For example, during proestrus, the activity of spinal aromatase as well as mER α are essential for the heterodimerization of MOR with KOR [32], the analgesic form of KOR [33], while at the same time spinal aromatase/mER α activity *does not* modulate spinal EM2 antinociception (although it does during diestrus) [26]. These differences imply that discrete pools of locally synthesized estrogens independently activate discrete populations of spinal mER α . This realization underscores the imperative to be cautious when associating particular CNS functionalities with circulating levels of ovarian steroids, and to consider cycle stage as a covariate in all investigations of nociception and opioid analgesic responsiveness in females.

1.6. Divergent mGluR₁ modulation of spinal EM2 antinociception

In addition to mER α and locally synthesized estrogens, mGluR₁ activation is also a prerequisite for suppression of i.t. EM2 analgesia during diestrus. This is reflected by the ability of spinal mGluR₁ blockade to unmask spinal EM2 antinociception [26]. Importantly, mGluR₁-coupled suppression of spinal analgesic responsiveness to i.t. EM2 does not result from the ability of mGluR₁ activity per se to promote hyperalgesia (independent of mERs and/or MOR), since spinal mGluR₁ blockade by itself does not lower nociceptive response thresholds (reflected by tail flick latency). Instead, mGluR₁ functionally associates with mER α to suppress spinal EM2 analgesia. This perspective is supported by the fact that mER α and mGluR₁ not only co-immunoprecipitate during diestrus, but that the magnitude of this co-immunoprecipitation diminishes during proestrus [26]. Notably, neither the activation of spinal mGluR₁ nor spinal mER α suppresses spinal EM2 antinociception during proestrus, the estrous cycle stage when the analgesia produced by i.t. EM2 becomes robust, comparable to that seen in male rats [26].

Many functions are modulated by mER α -mGluR₁ signaling. However, the outcomes of such signaling should not be extrapolated from one functional modality to the other. For example, in spinal cord of diestrous rats, mER α -mGluR₁ signaling suppresses i.t. EM2 antinociception, whereas in the medial preoptic nucleus estradiol-activated mER α directly stimulates mGluR_{1a}, which is responsible for the rapid activation of MOR, triggering female sexual receptivity [34]. The opposing direction of modulating MOR systems by mER α -mGluR₁ signaling, stimulatory in medial preoptic nucleus (during proestrus) and inhibitory in spinal cord (during diestrus), underscores the variable influence of stage of cycle as well as CNS region specificity. Had cycle stage been ignored, it is likely that the negative modulation of spinal EM2 antinociception by mER α -mGluR₁ would have gone undetected.

1.7. Transition from suppressive to facilitative modulation of spinal EM2 antinociception

The loss of spinal mER α -mGluR $_1$ suppression of i.t. EM2 antinociception is necessary but not sufficient for the emergence of spinal EM2 analgesic responsiveness during proestrus. Recruitment of additional signaling strategies is also required. The emergence of new signaling reveals the complexity that has evolved for gating spinal EM2 antinociception. This complexity is comprised of the ebb and flow of dynorphin/KOR signaling as well as the identity of the activator of mGluR $_1$, switching between mER α and glutamate.

1.8. Spinal EM2 antinociception requires spinal dynorphin/KOR activity

We previously reported that in addition to MOR, spinal dynorphin and KOR activities are also required for the robust spinal EM2 analgesia observed during proestrus [15,16]. This suggested that variability in spinal dynorphin release could be a critical determinant of the ability of intrathecally applied EM2 to produce antinociception. Indeed, spinal dynorphin release increases nearly twofold during proestrus compared to diestrus [22]. Moreover, the ability of blocking either mER α or mGluR $_1$ to unmask i.t. EM2 analgesia during diestrus results from the consequent increase in spinal dynorphin release and KOR activity [22]. Thus, insufficient spinal dynorphin/KOR signaling is one basis for the absence of spinal EM2 analgesic responsiveness during diestrus. This not only underscores the interdependence of two spinal opioid analgesic systems, but also provides a neurochemical foundation for the reported variable nociception across the reproductive cycle [29,31].

In addition to the disinhibition of spinal dynorphin release and KOR activity, which can be considered a derivative of the loss of suppressive modulation by mER α -mGluR $_1$ signaling, the emergence of robust analgesic responsiveness to i.t. EM2 also requires a switch in the nature of the endogenous mGluR $_1$ agonist. Paradoxically, mGluR $_1$ signaling, which suppresses spinal EM2 antinociception during diestrus [22], is essential for EM2 antinociception during proestrus. This qualitative shift in mGluR $_1$ modulation of EM2 analgesia results from (1) a change in the endogenous activator of mGluR $_1$ from mER α (during diestrus) to glutamate (during proestrus) [22,26], as well as (2) the partnering of mGluR $_1$ with mGluR $_{2/3}$ (both of which are present in a common spinal oligomer) and their co-activation [22] (Table 2). The importance of glutamate-activated mGluR $_1$ to spinal EM2 antinociception is shown by (1) the substantial reduction in spinal EM2 antinociception during proestrus following acute inhibition of spinal glutamate release (via riluzole) [22] (as was observed following spinal mGluR $_1$ blockade) and (2) the emergence of spinal EM2 antinociception during diestrus following the i.t. application of either glutamate itself (unpublished observations) or PDC [22] (a glutamate transport/reuptake inhibitor [35,36]) (Table 2).

The differential regulation of spinal EM2 analgesic responsiveness by mGluR $_1$, depending on its activation by either mER α or glutamate, suggests that spinal mGluR $_1$ signaling is subject to biased agonism – agonist-specific stabilization of receptor conformations, resulting in preferential activation of one of several possible receptor-coupled signaling pathways. In the case of mGluR $_1$, this is particularly relevant since mGluR $_1$ couples not only to G $_q$ [37], but also to G $_s$ [38,39] and G $_{i/o}$ [40,41]. The specific G protein coupling favored

by mGluR₁ could be significantly influenced by its activation by mER α (as occurs during diestrus) or glutamate (as occurs during proestrus).

1.9. Putative drug targets for harnessing endogenous opioid antinociception

It is well established that antinociceptive activity of endogenous opioid systems is quiescent under resting physiological conditions [42,43]. However, a variety of physiological [44] and behavioral stressors [[45], [46], [47], [48], [49], [50], [51]] elicit endogenous opioid antinociception. Whether or not pharmacological agents can also activate endogenous opioid antinociception remains an open question. The ability to reliably harness endogenous opioid antinociception would clearly be of considerable clinical value. Nevertheless, pharmaceutical companies have not actively pursued development of pharmacotherapies that enable tapping into endogenous opioid analgesic activity, due in large part to the absence of identified specific targets whose activity modulates the activity of endogenous opioid analgesic systems.

The success of designing pharmacotherapies for the purpose of harnessing endogenous spinal EM2 antinociception based on determinants of the analgesia elicited by i.t. EM2 requires a spinal anatomical organization that enables endogenous interactions among presumed regulatory components that parallel those that occur following the i.t. application of EM2. In this regard, it is noteworthy that there are spinal neurons coexpressing MOR, mER α mGluR₁, and aromatase which are apposed by EM2 varicosities [26], providing an anatomical basis for physiological modulation of endogenous spinal EM2 antinociception by mER α -mGluR₁ signaling. Additionally, vesicular glutamate transporter (VGLUT)-expressing (glutamatergic) terminals appose spinal dynorphin-expressing neurons that coexpress mGluR₁ and ER α [22].

This anatomical organization provides a cellular basis for cycling between ER α -activated and glutamate-activated endogenous mGluR₁ signaling over the estrous cycle. This organization would also enable modulation of spinal dynorphin release by glutamate, thereby coordinating glutamate activation of mGluR₁ with dynorphin release, both of which are prerequisites for spinal EM2 antinociception to be manifest.

Our studies thus suggest multiple potential spinal targets for unmasking spinal EM2 analgesia, particularly during diestrus and the corresponding menstrual cycle stage, around the time of menses [52] (summarized in schematic below). These would include antagonists selective for ER α /mGluR₁ and aromatase inhibitors, as well as KOR agonists and perhaps even i.t. glutamate itself (during menses, approximately equivalent to diestrus) to ameliorate the suppressive modulation of spinal EM2 analgesia. Additionally, drugs targeting contact points (“hot spots”) [[53], [54], [55], [56]] between components of the spinal oligomer containing MOR-KOR-mGluR₁-mGluR_{2/3}-aromatase-mER α could also be useful in either attenuating EM2 suppressive or facilitating EM2 analgesic signaling during menstrual cycle stages corresponding to diestrus and proestrus, respectively. Such drugs hold the promise of unleashing the prodigious antinociceptive potential of endogenous opioids, lessening the requirement for exogenous opioids and thereby reducing the risk of opioid abuse. These drugs would be expected to be particularly effective for treating chronic pain syndromes such as fibromyalgia, migraine and TMJ, which predominantly afflict women and are most

severe around the time of menses (comparable to diestrus in rats) when spinal EM2 antinociception is suppressed.

1.10. Consequences of undermanaging pain

Overall brain function can be harmed by chronic pain, which, if left untreated, can evolve into pain of much greater underlying pathophysiological complexity than the initial causes of the nociception. This transition can involve structural and functional changes in the nervous system [[57], [58], [59], [60]], which, as is the case with chronic back pain, includes loss of neocortical gray matter volume related to pain duration [61]. Chronic pain has also been associated with profound physiological consequences, which include altered mental states (e.g. [62]), sleep, and cardiovascular health (see [63] for review). Moreover, if left unaddressed, the neuroplastic changes that underlie this pain progression can become irreversible and ultimately untreatable. These considerations underscore the urgency for early and effective pain management.

1.11. Pain management and the prescription opioid abuse epidemic

Managing chronic pain, is complicated by concerns regarding addiction to and abuse of opioid analgesics, notwithstanding that they remain among the most effective pharmacotherapies for ameliorating many types of pain. This concern is magnified by the ongoing prescription opioid abuse epidemic that is ravaging society and the tens of thousands of deaths per year attributable to opioid overdose [64]. The dilemma this poses is how to continue to benefit from the substantial, virtually unparalleled pain-mitigating properties of opioids while eliminating addiction to them.

The medicinal 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 abuse. Endogenous opioids have not only been shown to mediate placebo analgesia [[1], [2], [3],6,7], but to also utilize the same neural mechanisms that mediate the opioid analgesia produced by narcotics [65]. Furthermore, it has been suggested that responsiveness to opioid analgesics can predict the magnitude of opioid placebo responsiveness, underscoring shared opioid mechanisms and the likely clinical utility of eliciting endogenous antinociception for chronic pain management.

Extrapolating from the exogenous, i.t. application of EM2, endogenous spinal opioid analgesia appears to be tightly regulated, cycling (in females) between activation of suppressive and facilitative modulatory systems. Strikingly, this ebb and flow correlates with specific stages of the estrous, and presumably menstrual, cycle. Antagonists/inhibitors of components of the suppressive modulatory system are likely to be viable drug targets for effectively unleashing endogenous opioid activity. Conversely, antagonists/inhibitors of the facilitative modulatory system are likely to worsen nociception/allodynia by lessening endogenous EM2 activity.

These considerations emphasize the need for a patient-specific medicine approach that includes stage of menstrual cycle as an important determinant of drugs with which to tap into endogenous antinociceptive capabilities. Utilization of drugs that harness endogenous

opioid antinociception in accordance with varying physiological state would open new possibilities for effective pain management.

We recently directly tested our prediction that components of the endogenous spinal opioid suppressive and facilitative modulatory systems could serve as drug targets for modulating endogenous opioid antinociception [66]. For this purpose, we utilized the spinal nerve ligation (SNL) chronic pain model [[67], [68], [69]]. Strikingly, all the i.t. treatments that unmask i.t. EM2 analgesic responsiveness in intact diestrous rats (blockade of spinal mER α /mGluR $_1$, inhibition of spinally synthesized estrogens) are also anti-allodynic in diestrous rats that had been subjected to SNL. In like fashion, mGluR $_1$ blockade, which substantially attenuates i.t. EM2 analgesic responsiveness during proestrus, worsened the SNL-induced allodynia during proestrus. These findings strongly suggest that the functionality of the endogenous spinal EM2 system, and likely other endogenous opioid systems, can significantly influence the severity of chronic neuropathic pain. This reveals pharmacological approaches that can be taken to harness endogenous opioid antinociception and the putative clinical utility of doing so (Scheme 1).

Abbreviations

CNS	central nervous system
DAMGO	[D-Ala ² ,N-Me-Phe ⁴ ,Gly-ol ⁵]-enkephalin
Dyn	dynorphin
EM2	endomorphin 2
i.t.	intrathecal
KOR	κ opioid receptor
LY341495	(2 <i>S</i>)-2-Amino-2-[(1 <i>S</i> ,2 <i>S</i>)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl)propanoic acid (mGluR $_{2/3}$ antagonist)
mERα	membrane estrogen receptor α
mGluR$_1$	metabotropic glutamate receptor 1
mGluR$_{2/3}$	metabotropic glutamate receptor 2/3
MOR	μ opioid receptor
MPP	1,3-Bis(4-hydroxyphenyl)-4-methyl-5-[4-(2-piperidinyloxy)phenol]-1H-pyrazole dihydrochloride (ER α -selective antagonist)
TMJ	temporomandibular joint dysfunction
VGLUT	vesicular glutamate transporter
YM298198	6-amino- <i>N</i> -cyclohexyl- <i>N</i> ,3-dimethylthiazolo[3,2- α]benzimidazole-2-carboxamide hydrochloride (mGluR $_1$ antagonist)

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Highlights

- Activating endogenous opioid systems can produce analgesia while avoiding opioid abuse.
- Opioid drugs and native opioid peptides have different profiles of action.
- Mechanisms mediating antinociception can differ over the reproductive cycle.
- Targeting spinal estrogenic and glutamatergic signaling in a physiological state-dependent manner can harness endogenous opioid analgesia.

Scheme 1. Schematic illustrating putative spinal targets for activating endogenous opioid antinociception in diestrous rats.

(A) Spinal dynorphin (Dyn) release (presynaptic Dyn neuron shown in schematic) is physiologically suppressed by locally synthesized estrogens acting via membrane ER α , which partners with mGluR $_1$ to restrict spinal EM2 analgesia. mER α , mGluR $_1$ and Aro are also coexpressed in spinal neurons that are apposed by EM2 (and presumably Dyn) varicosities. It is not known if mER α , mGluR $_1$ and Aro on the postsynaptic side are also involved in gating EM2 analgesic responsiveness. (B) Disinhibition of spinal EM2 analgesia is achieved by inhibiting spinal estrogenic signaling via either inhibiting aromatase (Aro), blocking mER α or mGluR $_1$ (depicted by red crosses). Any of these perturbations disinhibits dynorphin release (i.e., removes the inhibition resulting from mER α activation of mGluR $_1$), unveiling spinal EM2 analgesic responsiveness. (C) Disinhibition of spinal EM2 analgesia can also be achieved by switching the activator of mGluR $_1$ from membrane ER α to glutamate (Glut). This is achieved by either pharmacologically facilitating glutamate release or inhibiting its reuptake (depicted by a plus sign and red cross, respectively). We hypothesize that augmented synaptic glutamate diminishes the suppressive contribution of membrane ER α activation of mGluR $_1$ (depicted by the faded arrow between the two), thereby augmenting dynorphin release. Relationships among neurons depicted and components therein are informed by our recent publications (Liu, et al., Pain 2017 and Liu, et al., J. Neurosci. 2017).

Table 1.

Underpinnings of spinal MOR agonist-induced antinociception.

Opioid	Mediator		Antinociception	
	In diestrus	In proestrus	In diestrus	In proestrus
EM2	—	MOR & Dyn/KOR	None	Robust
Sufentanil/DAMGO	MOR	MOR	Robust	Robust
Morphine	MOR	MOR & Dyn/KOR	Robust	Robust

Table 2.

Drug targets that regulate spinal EM2 analgesia in female rat.

I.t. pre-treatment	<u>Effect on i.t. EM2 analgesia</u>	
	In diestrus	In proestrus
Vehicle (DMSO/Saline)	No effect	No effect
Block ER α (MPP)	Restore	No effect
Inhibit aromatase (Fadrozole)	Restore	No effect
Block mGluR ₁ (YM298198)	Restore	Block
Block mGluR _{2/3} (LY341495)	No effect	Block
Glutamate (Glut)	Restore	N/A
Block Glut reuptake (PDC)	Restore	No effect
Inhibit Glut release (Riluzole)	No effect	Block