Genetic behavioral screen identifies an orphan anti-opioid system

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Opioids target the μ-opioid receptor (MOR) to produce unrivaled pain management, but their addictive properties can lead to severe abuse. We developed a whole-animal behavioral platform for unbiased discovery of genes influencing opioid responsiveness. Using forward genetics in Caenorhabditis elegans, we identified a conserved orphan receptor, GPR139, with anti-opioid activity. GPR139 is coexpressed with MOR in opioid-sensitive brain circuits, binds to MOR, and inhibits signaling to heterotrimeric guanine nucleotide-binding proteins (G proteins). Deletion of GPR139 in mice enhanced opioid-induced inhibition of neuronal firing to modulate morphine-induced analgesia, reward, and withdrawal. Thus, GPR139 could be a useful target for increasing opioid safety. These results also demonstrate the potential of C. elegans as a scalable platform for genetic discovery of G protein–coupled receptor signaling principles.
Forward genetic screen identifies genes affecting behavioral sensitivity to opioids

The effects of opioids on tgMOR C. elegans and the molecular conservation of regulatory mechanisms prompted us to adopt this platform for an unbiased, forward genetic screen for regulators of opioid signaling (Fig. 2A). We focused on identifying mutants with increased opioid sensitivity to uncover negative regulators of MOR signaling.

Key to the design of our screen was the observation that a greater opioid response leads to faster paralysis and more rapid recovery. Thus, hypersensitive animals like tgMOR; rsbp-1 recover faster from the same drug dose than do tgMOR animals (Fig. 1, J and K). As a result, bulk segregation on plates was used to isolate hypersensitive mutants on the basis of their quicker recovery from opioid-induced paralysis and escape from the starting zone (Fig. 2A). A assay optimization with a mixture of tgMOR animals and hypersensitive tgMOR; rsbp-1 mutants showed that primary screening with morphine followed by secondary screening with fentanyl minimized false-positive rates (Fig. 2A).

For the full-scale screen, we mutagenized ~2500 tgMOR animals, evaluated ~600,000 progeny, and identified ~900 mutants with abnormal sensitivity to both morphine and fentanyl (Fig. 2B). Secondary evaluation in liquid thrashing assays with fentanyl eliminated false positives, identified mutants that lost opioid sensitivity, and confirmed a small number of hypersensitive mutants (Fig. 2B). We focused our efforts on comprehensive testing of opioid-induced behaviors for two mutants, tgMOR; bgg8 and tgMOR; bgg9. Both mutants had normal overall morphology.
and behavior in the opioid naïve state, but were paralyzed by fentanyl significantly faster than tgMOR worms (Fig. 2, C and D). Additional dose-response studies showed a leftward shift in fentanyl-induced paralysis, indicating that tgMOR; bgg8 and tgMOR; bgg9 mutants are hypersensitive to opioids (fig. S2).

We mapped genetic lesions causing hypersensitivity by combining whole-genome sequencing with phenotypic selection (fig. S3A). This process identified genomic regions of interest (3 to 5 Mb) that contained about six to eight different lesions per mutant. To determine which lesion caused opioid hypersensitivity, we used CRISPR-Cas9 to edit single mutations into candidate genes of tgMOR animals (fig. S3A).

For tgMOR; bgg8 animals, we identified a lesion in the calcium channel egl-19 that introduces a premature stop codon and likely results in loss of function (fig. S3B). CRISPR-Cas9 editing of the same egl-19 mutation into parental tgMOR animals confirmed that egl-19 affects opioid sensitivity (Fig. 2C and fig. S4, A and B). Notably, egl-19 is homologous to L-type Ca2+ channels in mammals, and extensive evidence indicates that L-type Ca2+ blockers potentiate the nociceptive properties of opioids in a clinical setting (28, 29). These observations demonstrate that our forward genetic screen identified conserved regulators of MOR signaling.

Another hypersensitive mutant, tgMOR; bgg9, contained a premature stop in frpr-13, which encodes an unstudied orphan GPCR (fig. S3C).

CRISPR-Cas9 editing of this lesion into tgMOR increased sensitivity to fentanyl, confirming that frpr-13 affects opioid sensitivity (Fig. 2D and fig. S4, C and D). Because the function of FRPR-13 is unknown, we further validated that it regulates opioid responses by transgenically expressing FRPR-13 in tgMOR; bgg9 mutants (fig. S3A). FRPR-13 expressed with the native frpr-13 promoter and MoS single-copy insertion (MosSCI) reversed the hypersensitivity of frpr-13 (bgg8) mutants back to a normal response (Fig. 2E and fig. S4, E and F). Similarly, hypersensitivity of frpr-13 (bgg8) mutants was reversed when FRPR-13 was pan-neuronally expressed with MosSCI (Fig. 2E). Collectively, these results indicate that the FRPR-13 receptor alters sensitivity to opioids at a behavioral level.

**FRPR-13/GPR139 negatively regulates MOR signaling**

Phylogenetic analysis revealed that FRPR-13 belongs to a large neuropeptide receptor group in C. elegans that is similar to two mammalian orphan GPCRs, GPR139 and GPR142 (fig. S5). GPR139 and GPR142 are in a distinct subfamily of class A orphan receptors (30). Given that nothing is known about FRPR-13 and there is no prior connection between GPR139 or GPR142 and opioid signaling, we explored the functional conservation of these receptors. We focused on GPR139 because it is expressed in the central nervous system, whereas GPR142 is predominantly found in the periphery (31, 32). Transgenic expression of human GPR139 in tgMOR; bgg9 worms with disrupted FRPR-13 lead to significantly reversed hypersensitivity to fentanyl (Fig. 2F). This indicates that GPR139 is a functional ortholog of FRPR-13, and GPR139 can inhibit MOR signaling in vivo.

We used a panel of assays to evaluate how GPR139 influences MOR signaling in mammalian human embryonic kidney (HEK) 293T cells. MOR activation drove rapid hyperpolarization of membrane potential upon reconstitution with the G protein–gated inwardly rectifying K+ (GIRK) channel (Fig. 3, A and B). Introduction of GPR139 cDNA in equivalent concentrations to MOR inhibited morphine-induced hyperpolarization, whereas overexpression of GPR139 in high amounts nearly abolished GIRK activation (Fig. 3, B and C). GPR139 communoprecipitated with MOR, indicating that these receptors can interact in a model cellular environment (Fig. 3D and fig. S6A). The relevance of this interaction in an endogenous context remains to be established. We detected significant reduction of MOR at the cell surface when GPR139 was expressed at high levels, suggesting that GPR139 can impede MOR trafficking (Fig. 3, E and F). Yet, at stoichiometric levels, GPR139 had no effect on surface localization of MOR, suggesting that GPR139 has other mechanisms to inhibit MOR (Fig. 3F). Indeed, at these lower stoichiometric levels, GPR139 promoted association of the signaling inhibitor β-arrestin with MOR (Fig. 3, G to I). This suggests that GPR139 has some constitutive activity that is sufficient to trigger β-arrestin recruitment. To further understand the implications of GPR139-MOR heteromerization and ensuing increased β-arrestin recruitment, we tested how GPR139
influenced MOR-mediated activation of G proteins in a bioluminescence resonance energy transfer (BRET) assay (Fig. 3J) (33). Morphine produced a rapid BRET response, reflecting rearrangement in Gαoβγheterotrimers induced by MOR activation (Fig. 3, K and L). Coexpression of GPR139 at low amounts inhibited MOR-induced G protein activation (Fig. 3, K and L, and fig. S6B). This inhibitory effect was more pronounced if GPR139 was expressed at high amounts owing to additional loss of MOR from the surface. Together, these results indicate that GPR139 can exert inhibitory effects on MOR in a cell-autonomous manner by affecting both receptor trafficking and signaling properties.

To probe the physiological relevance of inhibitory influences of GPR139 on opioid signaling in the mammalian nervous system, we used mouse models. GPR139 was expressed in brain regions implicated in opioid actions on reward, analgesia, and withdrawal (Fig. 4, A and E, and fig. S7) (31, 32). GPR139 was extensively coexpressed with MOR in a number of neuronal populations in these areas, most prominently in the medial habenula (MHB) and locus coeruleus (LC) (Fig. 4, A

Fig. 3. GPR139 inhibits MOR signaling. (A) Experimental design for evaluating MOR signaling through its effector GIRK. MOR activation leads to Gβγ subunit release, which opens GIRK channels to produce membrane hyperpolarization ($V_m$) that is measured with voltage-sensitive dye. (B) Coexpression of GPR139 inhibits MOR-mediated kinetics of membrane potential change in response to morphine (0.1 μM). (C) Quantification shows that GPR139 reduces morphine effects on $V_m$ amplitude. (D) Coimmunoprecipitation of MOR-FLAG and myc-GPR139 following their coexpression. (E) Experimental design for evaluating cell surface abundance of MOR. HiBiT-tagged MOR complements the LargeBiT (LgBiT) nanoluciferase enzyme only at the plasma membrane. (F) Quantification of the maximal cell surface content of HiBiT-MOR indicates that GPR139 inhibits MOR surface localization only at high expression levels (12 equivalents). (G) Experimental design for evaluating agonist-induced β-arrestin recruitment to MOR. Recruitment of β-arrestin2-LgBiT to SmBiT-MOR generates a functional nanoluciferase enzyme. (H) Effect of GPR139 coexpression on the kinetics of β-arrestin2-LgBiT recruitment induced by DAMGO (10 μM). (I) Quantification shows that low-level GPR139 coexpression increases the extent of β-arrestin2 recruitment to MOR. (J) Experimental design for evaluating MOR signaling to G proteins using a BRET assay, which measures MOR-mediated release of Gβγ subunits. (K) Effect of GPR139 coexpression on the kinetics of G protein activation by MOR in response to morphine (1 μM) application.
and E; fig. S7; and table S1). To test the role of GPR139 in opioid modulation, we obtained Gpr139 knockout mice (Gpr139< sup >−/−</sup >, fig. S8). We performed patch-clamp recordings of MHb neurons in brain slices with drugs that block synaptic communication and circuit activity. In slices from Gpr139<sup>+/−</sup> animals, MOR activation resulted in dose-dependent inhibition of spontaneous firing (Fig. 4, B and C). Firing of MHb neurons from Gpr139<sup>−/−</sup> mice was significantly reduced by low-level MOR activation, which did not cause an effect in Gpr139<sup>+/−</sup> neurons (Fig. 4, B and C). Furthermore, Gpr139<sup>−/−</sup> neurons showed more pronounced net inhibition by DAMGO, a synthetic enkephalin-mimetic peptide (Fig. 4D and fig. S9, A and B). Recovery upon drug washout was delayed in Gpr139<sup>−/−</sup> neurons, which indicates greater susceptibility to opioid inhibition (fig. S9C). Hypersensitivity to morphine-induced inhibition of firing in LC neurons also occurred in Gpr139<sup>−/−</sup> mice (Fig. 4, F and G). GPR139 ablation resulted in increased basal firing rates selectively in LC but not MHb neurons (fig. S9D). These findings indicate that GPR139 may counteract MOR cell-autonomously in endogenous physiologically relevant neuronal settings as well as reconstituted systems.

**GPR139 modulates behavioral responses to opioids**

To understand how GPR139 influences opioid actions in vivo, we evaluated mouse behavior. Deletion of GPR139 had no overt effects on animal health and body composition (fig. S10, A to C). Gpr139<sup>−/−</sup> mice also had normal baseline learning (Fig. 5A), nociception (Fig. 5B), locomotor activity (fig. S10D), habituation to an unfamiliar environment (fig. S10E), and motor coordination (fig. S10F). However, responses of Gpr139<sup>−/−</sup> mice to morphine were increased. When tested in a conditioned place preference (CPP) paradigm, Gpr139<sup>−/−</sup> mice showed augmented responses to the rewarding effects of morphine (Fig. 5A), in agreement with increased opioid sensitivity of Gpr139<sup>−/−</sup> MHb neurons (Fig. 4, B to D), a region involved in drug reward (34). Similarly, Gpr139<sup>−/−</sup> mice exhibited significantly increased morphine analgesia in thermal (Fig. 5, B and C, and fig. S11A) and mechanical (fig. S11B) pain paradigms. This augmentation was evident from increases in both maximal response and effect duration across multiple morphine doses (Fig. 5, B and C, and fig. S11, A and B). Thus, deletion of GPR139 broadly increases sensitivity to the acute effects of morphine. Termination of chronic morphine administration caused lower somatic withdrawal in mice lacking GPR139, which was evident across a spectrum of measures (Fig. 5D and fig. S12). The diminished withdrawal observed in Gpr139<sup>−/−</sup> mice may be related to observed changes in baseline firing rate seen in Gpr139<sup>−/−</sup> LC neurons (Fig. 4F and fig. S9D), a neuronal population involved in opioid withdrawal (35).

To test the translational relevance of our findings, we examined the effects of JNJ-63533054, a surrogate ligand that facilitates GPR139 actions (32). Administration of JNJ-63533054 diminished morphine analgesia in a dose-dependent fashion in both the thermal and mechanical pain paradigms (Fig. 5E). These effects were not observed in Gpr139<sup>−/−</sup> mice, indicating specificity of JNJ-63533054 actions (fig. S13). To determine if activating GPR139 affects reward,
Fig. 5. GPR139 controls behavioral sensitivity of mice to opioid administration. (A) Conditioned place preference paradigm showing increased reward in Gpr139<sup>−/−</sup> mice. (B) Hot plate assay showing increased dose-dependent, antinociceptive effects of morphine in Gpr139<sup>−/−</sup> mice. (C) Gpr139<sup>−/−</sup> animals had increased duration of morphine analgesia in a hot plate assay. (D) Gpr139<sup>−/−</sup> mice had decreased behavioral responses and weight loss due to naloxone-precipitated somatic withdrawal after chronic morphine exposure. The global score reflects an aggregate measure of several withdrawal signs (diarrhea, jumps, dog shakes, paw tremor, back walking, tremor and ptosis). (E) Augmentation of GPR139 function by JNJ63533054 decreases analgesia induced by morphine (10 mg/kg) across pain models. (F) Activation of GPR139 by JNJ63533054 inhibits morphine intake (0.3 mg/kg per infusion) in self-administration task. (G) Quantification of JNJ63533054 effects on morphine self-administration. Significance was tested using two-way ANOVA or Student’s t-test. Animal numbers for each test provided in the materials and methods. ***p < 0.001, **p < 0.01, *p < 0.05.

we examined the effects of JNJ-63533054 in a morphine self-administration paradigm. Following escalation of morphine intake, wild-type mice were divided into two groups with alternating exposure to JNJ-63533054. Administration of drug suppressed morphine intake (Fig. 5F and fig. S14). The effect of JNJ-63533054 was dose dependent and completely reversible upon cessation of exposure (Fig. 5F and fig. S14). Overall, these in vivo results indicate that GPR139 negatively regulates a number of responses to acute opioid exposure and potentiates withdrawal from chronic opioid administration.

Discussion

We developed a C. elegans behavioral platform for the unbiased genetic discovery of GPCR signaling modulators. Transgenic GPCR expression endows animals with the ability to respond to a foreign chemical modality, akin to chemogenetic approaches used to interrogate mammalian circuitry and behavior (36). The platform displays cardinal features of behavioral responses to receptor activation, allowing phenotypic interrogation of signaling pathways using intact neuronal circuitry in vivo. Use of behavior as an ultimate read-out provides a high degree of relevance and potential translational validity. Characterization of tgMOR C. elegans revealed cross-species conservation of critical GPCR signaling elements. This transgenic platform also demonstrated utility in uncovering the biology of an opioid receptor signaling network. The scalable nature of our screens may permit further exploration of signaling mechanisms for GPCRs of interest. Additionally, this approach could be adapted for different neuronal circuitry, behavioral readouts, and other GPCRs, thereby expanding potential opportunities for discovery.

Using forward genetic screening, we identified an evolutionarily conserved orphan receptor system with anti-opioid activity: FRPR-13 in C. elegans and its mammalian ortholog GPR139. Although the full spectrum of GPR139 effects on cellular physiology and mechanisms of suppressing MOR action remain to be elucidated, our examination indicates that some of these actions involve direct inhibitory influences of GPR139 on MOR signaling. Opposing cross-talk betweenGPCRs is an intriguing concept (37), and our study now adds the poorly understood GPR139 orphan receptor to a growing realm of molecules that oppose MOR (38–40). Notably, α-melanocyte stimulating hormone (a peptide derived from the same precursor as the MOR ligand, β-endorphin) was reported as one endogenous ligand for GPR139 (41). This further argues for the physiological importance of the GPR139–MOR connection and indicates that GPR139 might affect homeostatic control of the endogenous opioid signaling system. Whether GPR139 modulates endogenous opioid function remains to be determined.

Our results suggest that GPR139 could potentially be exploited pharmacologically to increase the safety and efficacy of opioid pharmacotherapy. Although our study focused on the anti-opioid effects of GPR139, widespread expression of GPR139 in the nervous system may indicate that this orphan receptor has additional roles in shaping neuronal physiology independent of MOR.

Materials and methods

For detailed description of all procedures and methods, refer to the supplementary materials.

REFERENCES AND NOTES

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Data and materials availability: All data are available in the manuscript or the supplementary materials. Reagents developed in this study are freely available upon request.

SUPPLEMENTARY MATERIALS
science.sciencemag.org/content/365/6459/1267/suppl/DC1
Materials and Methods
Figs. S1 to S14
Table S1
References (42–44)
Movies S1 to S10

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Worms yield opioid receptor insight

The µ-opioid receptor (MOR) is the target of pain-reducing drugs, including morphine and the potent synthetic opioid fentanyl. Better understanding of the receptor system is needed to suppress potentially deadly side effects and manage addiction potential. Wang et al. used a screen in the worm Caenorhabditis elegans to find genes that influenced MOR function (see the Perspective by Mercer Lindsay and Scherrer). They found another receptor called GPR139, loss of which enhanced effects of morphine in mice but reduced withdrawal effects. GPR139 could be a target to improve safety or efficacy of opioid therapy.

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