Research Paper

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Group II mGluRs suppress hyperexcitability in mouse and human nociceptors

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Abstract

We introduce a strategy for preclinical research wherein promising targets for analgesia are tested in rodent and subsequently validated in human sensory neurons. We evaluate group II metabotropic glutamate receptors, the activation of which is efficacious in rodent models of pain. Immunohistochemical analysis showed positive immunoreactivity for mGlu2 in rodent dorsal root ganglia (DRG), peripheral fibers in skin, and central labeling in the spinal dorsal horn. We also found mGlu2-positive immunoreactivity in human neonatal and adult DRG. RNA-seq analysis of mouse and human DRG revealed a comparative expression profile between species for group II mGluRs and for opioid receptors. In rodent sensory neurons under basal conditions, activation of group II mGluRs with a selective group II agonist produced no changes to membrane excitability. However, membrane hyperexcitability in sensory neurons exposed to the inflammatory mediator prostaglandin E2 (PGE_2) was prevented by (2R,4R)-4-aminopyrrolidine-2,4-dicarboxylate (APDC). In human sensory neurons from donors without a history of chronic pain, we show that PGE_2 produced hyperexcitability that was similarly blocked by group II mGluR activation. These results reveal a mechanism for peripheral analgesia likely shared by mice and humans and demonstrate a translational research strategy to improve preclinical validation of novel analgesics using cultured human sensory neurons.

Keywords: Human, Dorsal root ganglia, Physiology, Metabotropic, Glutamate, Sensitization

1. Introduction

Concern has grown over the frequency with which promising analgesic candidates fail to show efficacy in clinical trials, yet reasons for the lack of success have been difficult to establish.[32,51] One obstacle limiting translation may be the often erroneous assumption that results from animal models accurately predict drug efficacy in humans. Because of the inherent challenges in obtaining viable neural tissues from humans, testing species differences has not been considered feasible. However, we recently reported progress in using uninjured dorsal root ganglia (DRG) from adult human donors without chronic pain to characterize the physiological properties of individual human sensory neurons.[14] In this study, we functionally examine a potential analgesic at the level of the sensory neuron in a rodent model and validate its efficacy in human sensory neurons.

Group II metabotropic glutamate receptors (mGlu2 and mGlu3) are 7 transmembrane domain G protein-coupled receptors that canonically activate the Gi signaling pathway.[21] In several rodent models of inflammatory and neuropathic pain, pharmacological activation of group II mGluRs reduced nocifensive behaviors.[33,35,43,54] Immunoreactivity for group II mGluRs has been observed in rodent DRG neurons,[4,5] and cutaneous administration of a group II mGluR agonist suppressed capsaicin-evoked activity in nociceptors, indicating a peripheral mechanism of action.[3,16,53] Interestingly, pharmacological inhibition of peripheral group II mGluRs prolonged hyperalgesia and nociceptor activity, which suggests that group II mGluRs act endogenously to reverse hypersensitivity.[6,54]

Several animal studies have taken advantage of the endogenous antihyperalgesic action of group II mGluRs by inducing therapeutic transcriptional upregulation of group II mGluRs in DRG.[8,12,56,57] Oral administration of the dietary supplement and group II mGluR epigenetic modulator acetyl-L-carnitine produced analgesic effects in humans with diabetic or HIV-related peripheral neuropathies.[11,27] Additionally, enhanced endogenous activation of group II mGluRs with oral N-acetyl cysteine reduced nocifensive behaviors in mice,[1] and laser-evoked pain ratings in humans.[46]

Direct, pharmacological activation of group II mGluRs has been explored in clinical trials, and a satisfactory safety profile has been established for these drugs.[39] However, clinical trials have yet to examine the effects of direct group II mGluR activation for pain relief. We examine the expression of group II mGluRs in the peripheral nervous system of mice and humans. We then determine the effect of direct activation of group II mGluRs on membrane excitability from sensory neurons isolated from mouse and human DRG in a series of parallel experiments.

2. Materials and methods

2.1. Animals

All experiments were conducted in accordance with the National Institute of Health guidelines and received the approval of the

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

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Animal Care and Use Committee of Washington University School of Medicine. Eight- to 12-week old littermate mice (C57BL/6, Jackson lab) were housed on a 12-hour light–dark cycle and allowed ad libitum access to food and water. Knockout mice (mGlu2$^{-/-}$) were generously provided by Eli Lilly for immunohistochemistry.

2.2. Immunohistochemistry

For immunohistochemistry, mice were deeply anesthetized (ketamine–xylazine–acepromazine) and perfused with ice cold saline solution followed by 4% paraformaldehyde. Glabrous skin from the hind paw was dissected before perfusion and immersion-fixed in Zamboni fixative for 4 to 6 hours, rinsed in PBS and then cryoprotected in 30% sucrose. The DRG and spinal cord were removed and stored in 30% sucrose and then embedded in cutting medium and sectioned at 30 μm on a cryostat. Sections were collected on slides. Antibodies were anti-mGlu2 (1:200-400; Sigma, St Louis, MO, SAB4501318), anti-βIII tubulin (1:1000; Covance, Princeton, NJ), anti-calcitonin gene-related peptide (anti-CGRP) (1:400; AbD Serotec (now Bio-Rad), Raleigh, NC), and Alexa-fluor 555 or 488 (1:400; Invitrogen, ThermoFisher Scientific, Waltham, MA). Isolectin B4-conjugated to Alexa-fluor 568 (1:400; Invitrogen) was used to identify versican-positive neurons. For human tissues, DRG were postfixed in 4% paraformadehyde then stored in 30% sucrose until sectioned. Autofluorescence from endogenous lipofuscin prevented clear labeling with fluorescent antibodies, therefore diaminobenzidine horseradish peroxidase staining was used (Vector Labs, Burlingame, CA).

2.3. RNA-seq analysis

Human L2 DRG from 3 female tissue donors free of pain-related disease and ranging in age from 40 to 50 years were obtained through AnaBios, Inc (San Diego, CA). Poly-A+ RNA was sequenced from a 75-bp paired-end library on an Illumina sequencer by ActiveMotif (Carlsbad, CA). Results were integratively analyzed with the publicly available human fetal spinal cord from the ENCODE project[13] (Replicate 1 [male]: ENCFF001RNA, ENCFF001RNB; Replicate 2 [female]: ENCFF001RNC, ENCFF001RND) RNA-seq data. Publicly available RNA-seq data were obtained for mouse adult female DRG (strain C57BL/6: GEO data sets GSM1150934, GSM1150935),[22] and adult female spinal cord (strain C57Bl/6J: GEO data sets GSM1103369, GSM1103370).[7] Paired-end data sets were converted to single-end data sets by merging read libraries. At least 2 biological replicates for each tissue type thus obtained were mapped to the reference genome and transcriptome, using the Tophat-Cufflinks pipeline.[45] Quantification of RNA abundance was performed using the Cuffdiff tool in the Tophat-Cufflinks toolkit, using the "classic" normalization mode.[45] Abundances are reported for each gene in reads per kilobase per million mapped reads (RPKMs). Reference genomes and transcriptomes used for human and mouse RNA-seq mapping were NCBI hg19 + Gencode v14, and NCBI mm10 + Gencode vM4, respectively.[24] Data from RNA-seq experiments will be made available at the publicly accessible database of Genotypes and Phenotypes: http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs001158.v1.p1.

2.4. Cell culture

Wild-type C57/B6 mice were killed by decapitation, and the DRG was removed and incubated at (37°C, 5% CO_2) in 3 mL Ca^{2+}/Mg^{2+}-free Hank buffered saline solution containing 10 mM HEPES for 20 minutes with 45 U papain (Worthington, Lakewood, NJ) and then for 20 minutes with collagenase (1.5 mg/mL, Sigma). The DRG were triturated and then passed through a 40-μm filter and the dissociated cells plated on poly-D-lysine and collagen coated glass coverslips. Cells were cultured overnight in Neurobasal A media supplemented with B27, 100 U/mL penicillin/streptomycin, 2 mM GlutaMAX, and 5% fetal bovine serum (Gibco). Experiments were performed within 24 hours of plating.

Dorsal root ganglia from consented US donors were acquired by AnaBios, Inc., or through Mid-America Transplant Services (MTS, Saint Louis, MO) and prepared as described previously.[14] Detailed protocols for the preparation and experimental use of human sensory neurons are available.[47] Briefly, the DRG were dissected to remove connective tissue and fat. The ganglia were enzymatically digested and mechanically dissociated, and then cells were seeded on glass coverslips coated with poly-D-lysine. Cells were maintained in culture at 37°C with 5% CO_2 in DMEM F-12 (Lonza, Allendale, NJ) supplemented with 10% horse serum (ThermoFisher Scientific, Rockford, IL), 2 mM glutamine, 25 ng/mL hNGF (Cell Signaling Technology, Danvers, MA), 25 ng/mL GDNF (PeproTech, Rocky Hill, NJ), and penicillin/streptomycin (ThermoFisher Scientific). Human DRG neurons were incubated in culture for 3 days before recording.

2.5. Electrophysiology

Neurons from mice and humans were tested in an external recording solution consisting of the following (in mM): 145 NaCl, 3 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 7 glucose, and 10 HEPES, adjusted to pH 7.4 with NaOH and 305 mOsm with sucrose. Borosilicate, filamented glass electrodes with 2 to 5 MΩ resistance (Warner Instruments, Hamden, CT) contained internal solution (in mM): 130 K-gluconate, 5 KCl, 5 NaCl, 3 Mg-ATP, 0.3 EGTA, 10 HEPES, adjusted to pH 7.3 with KOH and 294 mOsm with sucrose. After gigaseal and break-in, neurons were given a series of protocols to determine membrane excitability. For acute bath application, drugs (PGE_2, Sigma; (2*R*,4*R*)-4-aminopyrrolidine-2,4-dicarboxylate (APDC), Tocris) were diluted in external solution. For the incubation protocol, PGE_2 or PGE_2 plus APDC were added to the media 30 minutes before recordings, which were performed in external solution containing the same concentrations of drug. Neurons were recorded within 30 minutes of placement in the recording chamber. Input resistance was calculated as ΔV/ΔI (using a 30- to 200-pA negative current injection). Rheobase was established from the 1-second step current pulse at which the first action potential was triggered. For threshold, action potentials were evoked using a series of increasing 1-second ramp current injections. For latency, the first action potential was measured from a 1-second, 150-pA ramp. The first action potential of a train was used to determine the threshold, defined as the voltage at which the first derivative of the membrane potential increased by 10 V/s. Dorsal root ganglia neurons were viewed using an Olympus BX-50 epifluorescence microscope. Data were collected with a HEKA EPC 10 amplifier, digitized at 20 kHz, and recorded on a PC running Patchmaster software (v2x-71). Series resistance was kept below 10 MΩ in all recordings, and only cells with a diameter of 32 μm (mouse) or 60 μm (human) or less were studied.

2.6. Statistics

Electrophysiology results are presented as mean ± SEM. Data were analyzed offline with Igor Pro (WaveMetrics, Portland, OR) using custom-written macros. Data organization and statistical analysis were performed using Microsoft Excel and Prism 6

(GraphPad, La Jolla, CA). Analysis of multiple groups was by analysis of variance and pairwise comparisons by paired or unpaired *t* tests as appropriate. Significance was taken at $P < 0.05$.

3. Results

3.1. Expression of mGlu2 in the peripheral nervous system

Somata within the mouse DRG and epidermal fiber terminals immunoreactive for an mGlu2-specific antibody were identified (**Fig. 1**). One-quarter of all DRG neurons were clearly immunostained with anti-mGlu2. Of mGlu2$^+$ neurons, 27.8 ± 2.3% were also positive for CGRP, a marker for small-diameter peptidergic sensory neurons, and 6.3 ± 4.5% of mGlu2$^+$ neurons coexpressed IB4, a marker for nonpeptidergic sensory neurons (**Fig. 1A, B**). Also, mGlu2 immunoreactivity was observed in 23.2 ± 5.1% of the CGRP population, and 5.8 ± 3.7% of the IB4 population of DRG neurons. Immunoreactivity for mGlu2 was also identified in terminal fibers within the footpad epidermis (**Fig. 1C**), and mGlu2$^+$ fibers accounted for 29% of the total fibers identified with the pan-neuronal marker β3-tubulin (**Fig. 1D**). Neurons positive for mGlu2 immunofluorescence possessed diameters ranging from 18 to 56 μm with a mean of 34.3 ± 0.7 μm, and 39.8% of mGlu2$^+$ neurons were ≤ 30 μm (**Fig. 1D**). To determine whether mGlu2 was also present in human primary afferent neurons, fixed human DRG were sectioned and stained using the same mGlu2 antibody. Dorsal root ganglia sections from both adult and neonatal human showed positive immunoreactivity for mGlu2 in both small and larger somata, indicating persistent expression throughout human life (**Fig. 1E-G**).

We further observed mGlu2-specific immunoreactivity appearing most densely in laminae III and IV of the mouse spinal cord dorsal horn (**Fig. 2**). Interestingly, little overlap was observed with expression of CGRP-positive fibers in lamina I (**Fig. 2A**) or with IB4-postive fibers in lamina II (**Fig. 2B**), suggesting selective targeting to the deeper dorsal horn by the central processes of mGlu2$^+$ sensory neurons. We confirmed that spinal cord tissue from mGlu2 knockout mice did not exhibit mGlu2 immunoreactivity within the dorsal horn (**Fig. 2C**).

3.2. RNA-seq for group II mGluRs

Next, we quantified the gene transcripts for mGlu2 (*GRM2*) and mGlu3 (*GRM3*) by examining the expression of *GRM2* and *GRM3* from RNA-seq data acquired from the DRG of 3 human donors and compared this with the abundance of RNA from public RNA-seq data obtained from reference mouse and human RNA-seq databases. Both human and mouse DRG expressed *GRM2* and *GRM3. GRM2* exhibited more than double the reads of *GRM3* in mouse DRG, but this difference was less prevalent in human (**Table 1**). We observed that human DRG possessed fewer *GRM2* and *GRM3* reads overall compared with the mouse DRG. To determine the potential relevance of these quantities for human analgesia we examined the abundance of the gene products for Mu (*OPRM1*) and Delta (*OPRD1*) opioid receptors, both of which are functionally significant in the clinical control of pain and could serve as anchor points.[44,49] Reads from these opioid G protein-coupled receptors indicated expression at similar levels to the group II mGluRs, which suggests that the quantities of *GRM2* and *GRM3*

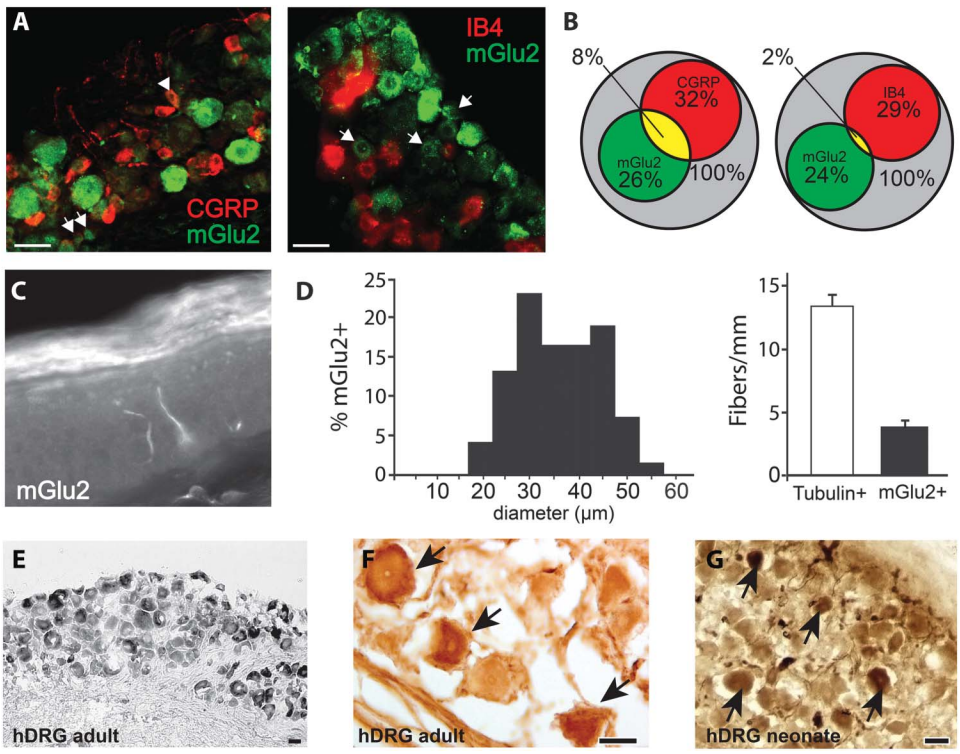

Figure 1. Anatomical localization of mGlu2 in mouse and human sensory neurons. (A) Anti-mGlu2 immunoreactivity in the mouse dorsal root ganglia (DRG) and coexpression with calcitonin gene-related peptide (CGRP) (arrowheads) and IB4. (B) Venn diagram showing the overlap and percentages of mGlu2$^+$, CGRP$^+$, and IB4$^+$ somata in the DRG (n = 367–389 total DRG neurons from 4 DRG from 3 animals). (C) mGlu2-IR fibers extend into the epidermis of the mouse footpad. (D) Size distribution of mGlu2-IR somata (34.3 ± 8.5, mean ± SD). mGlu2$^+$ fibers accounted for 29% of total (tubulin-positive) fibers in the epidermis. Data from 3 animals, one DRG or footpad quantified per animal. (E-G) Human adult and neonatal DRG neurons immunoreactive for mGlu2 stained with diaminobenzidine. All scale bars = 50 μm.

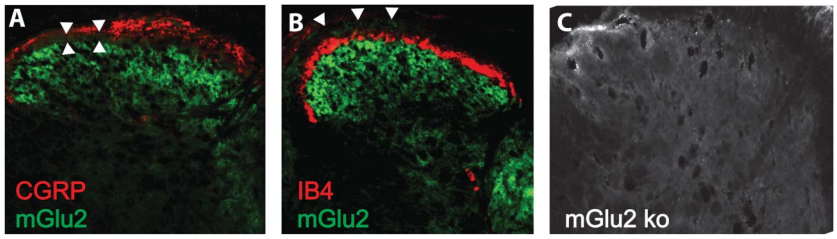

Figure 2. Anatomical localization of mGlu2 in the mouse spinal cord. (A) Expression of mGlu2 immunoreactivity in laminae III and IV shows little overlap with calcitonin gene-related peptide in the mouse dorsal horn (arrowheads). (B) Expression of mGlu2 immunoreactivity shows little overlap with IB4. (C) Absence of mGlu2 immunoreactivity in the mGlu2 knockout mouse.

could be sufficient for analgesic effects. Additional analyses of transcripts from the spinal cord are reported in **Table 1**.

3.3. Block of peripheral sensitization with group II mGluRs

The presence of both mRNA and protein for group II mGluRs in mouse and human DRG prompted us to investigate whether activation of group II mGluRs could modulate membrane excitability and block peripheral sensitization, a mechanism for hyperalgesia. In dissociated primary sensory neurons from the mouse, activation of group II mGluRs with the agonist APDC under basal conditions produced no changes in the membrane resting potential (**Fig. 3A**) or input resistance (**Fig. 3B**). In contrast, bath application of PGE_2, an inflammatory mediator that produces hyperexcitability of sensory neurons in part by increasing calcium and tetrodotoxin-resistant sodium currents,[23,28] reduced input resistance. This effect was reversed by APDC (**Fig. 3C**).

To further examine the effects of group II mGluR activation on membrane excitability, we quantified the rheobase, action potential threshold, and initial action potential latency (**Fig. 3D**). The addition of APDC produced no changes to excitability compared with vehicle-treated sensory neurons in both subsets of small- or large-diameter sensory neurons (**Fig. 3E, F**). However, sensory neurons ≤32 μm in diameter coincubated with PGE_2 and APDC exhibited significantly reduced membrane excitability compared with neurons incubated with PGE_2-alone (**Fig. 2G**). PGE_2-alone produced long-lasting, ongoing discharge in only 6% of mouse neurons. These results indicate that activation of group II mGluRs prevents the sensitization of rodent sensory neurons by the inflammatory mediator PGE_2, but has no effect on membrane excitability under basal (noninflammatory) conditions.

The question of whether the analgesic effect of group II mGluRs translates to humans depends critically on whether the receptor operates through similar mechanisms in human sensory neurons as it does in the mouse. To test this question, we extracted intact human DRG from donors without a history of chronic pain and used whole-cell patch-clamp techniques to measure neuronal excitability in vitro. We first tested whether PGE_2 could directly sensitize human DRG neurons (**Fig. 4A**). Indeed, PGE_2 lowered rheobase and hyperpolarized action potential threshold (**Fig. 4B**). Some human cells produced a transient discharge to PGE_2, but we detected no long-lasting, ongoing discharge. To determine whether the antihyperalgesic effects of group II mGluRs previously demonstrated in mice are mechanistically similar in human sensory neurons, APDC was bath-applied to human dorsal root ganglia (hDRG) neurons. Similar to mouse sensory neurons, the resting membrane potential of naive human DRG neurons was unaffected by activation of group II mGluRs with APDC (**Fig. 4C**). Nor did application of APDC alter input resistance. In contrast, the presence of APDC prevented PGE_2-induced hyperexcitability indicated by unchanged rheobase and action potential threshold compared with the naive state (**Fig. 4D, E**). Thus, activation of group II mGluRs in human sensory neurons blocks hyperexcitability but did not change basal membrane excitability.

4. Discussion

Prostaglandins and other inflammatory mediators increase the activity of peripheral fibers and generate pain, itch, and hyperalgesia in humans and can produce a transient discharge in human DRG neurons.[14,34,41] In this study, we show that PGE_2 alters membrane excitability in cultured human sensory neurons from donors without chronic pain by lowering rheobase and the action potential threshold. Furthermore, we show that the group II mGluR agonist APDC prevents the hyperexcitability induced by exposure to PGE_2 in the mouse and confirm this observation in human sensory neurons. The human mGlu2 receptor shares >90% sequence homology with rodents, suggesting evolutionary pressure for its maintenance but also the possibility for important functional differences between species.[18] The present

Table 1

RNA-seq of group II mGluRs and opioid receptors in mice and humans.

Gene	hDRG 1	hDRG 2	hDRG 3	hDRG ($\bar{X}$ ± SEM)	hSC	mDRG	mSC
GRM2	0.14	0.44	0.14	0.24 ± 0.1	0.61	1.11	1.09
GRM3	0.08	0.27	0.24	0.20 ± 0.06	17.68	0.44	7.34
OPRM1	0.11	0.51	0.55	0.39 ± 0.14	7.16	1.83	0.98
OPRD1	0.03	0.6	0.14	0.26 ± 0.17	0.39	2.70	2.10

Numbers are shown in reads per kilobase per million mapped reads (RPKMs) for each of the tissues listed. hDRG samples are L2 level from 3 female donors. hSC is from pooled fetal samples. mDRG and mSC are from adult female samples on C57Bl/6J background.

GRM2 → *mGlu2*; *GRM3* → *mGlu3*; *OPRD1* → δ opioid receptor; *OPRM1* → μ opioid receptor.

hDRG, human dorsal root ganglia; hSC, human spinal cord; mDRG, mouse dorsal root ganglia; mSC, mouse spinal cord.

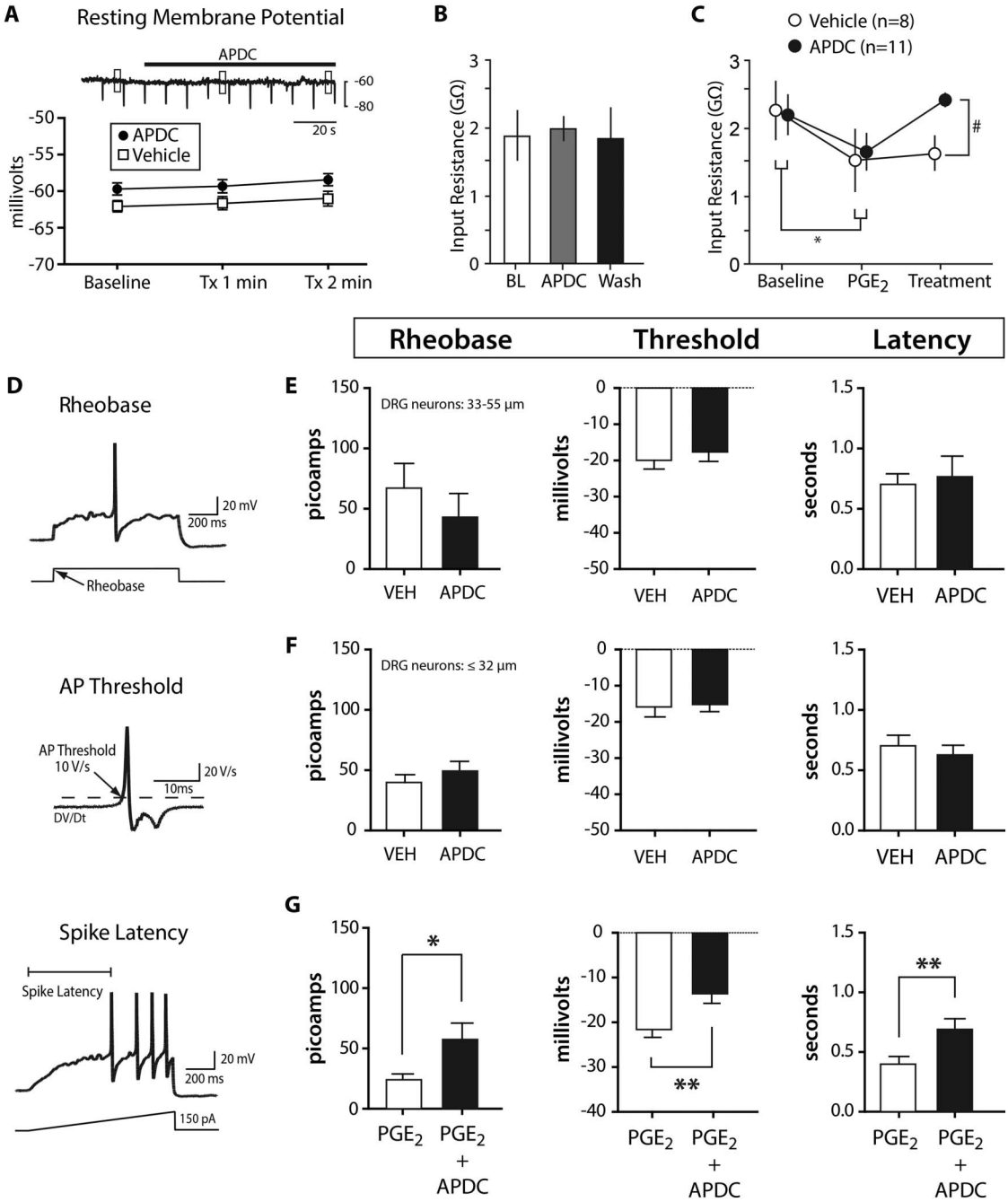

Figure 3. Activation of group II mGluRs blocks PGE_2-mediated hyperexcitability in the mouse. (A) APDC (1 μM) did not change the resting membrane potential. Boxes over trace show time points used to calculate means. Nor was input resistance changed by APDC (n = 8). Tx = treatment with APDC or vehicle (B). (C) Input resistance was significantly reduced by application of PGE_2 (1 μM), and the addition of APDC returned input resistance to baseline (2-way repeated-measures analysis of variance, Tukey posttest; within $^*P < 0.05$, between $\#P < 0.05$; n = 8–11). (D) Schematic for measuring rheobase, threshold, and latency. (E, F) Excitability was unchanged by APDC compared with vehicle in medium-large dorsal root ganglia (DRG) neurons (>32 μm) and in small DRG neurons (≤32 μm); n = 8 to 11. (G) Compared with PGE_2-alone, coincubation with APDC resulted in significantly higher rheobase, threshold, and latency in DRG neurons ≤32 μm (unpaired *t* test $^*P < 0.05$; $^{**}P < 0.01$; n = 13-15).

results demonstrate unprecedented preclinical functional and translational validation of a candidate analgesic target in humans. To extend these observations, we established that immunoreactivity for the mGlu2 receptor is present in the peripheral nervous system and that group II mGluR gene transcripts are expressed at functionally significant levels in both mice and humans. Interestingly, the activation of group II mGluRs under noninflammatory conditions produced no detectable changes to membrane excitability in either species, which suggests that group II mGluRs selectively counter hyperexcitability without modulating basal nociceptor excitability.

Previous work demonstrated that application of APDC into the rodent skin reduced the duration and magnitude of inflammatory pain responses, indicating a peripheral mechanism for the analgesic action.[16,54] Group II mGluR activation also prevented PGE_2-induced potentiation of capsaicin-evoked calcium responses,[53] and reduced capsaicin-evoked nociceptive fiber activity, which suggests that the antinociceptive effect may be

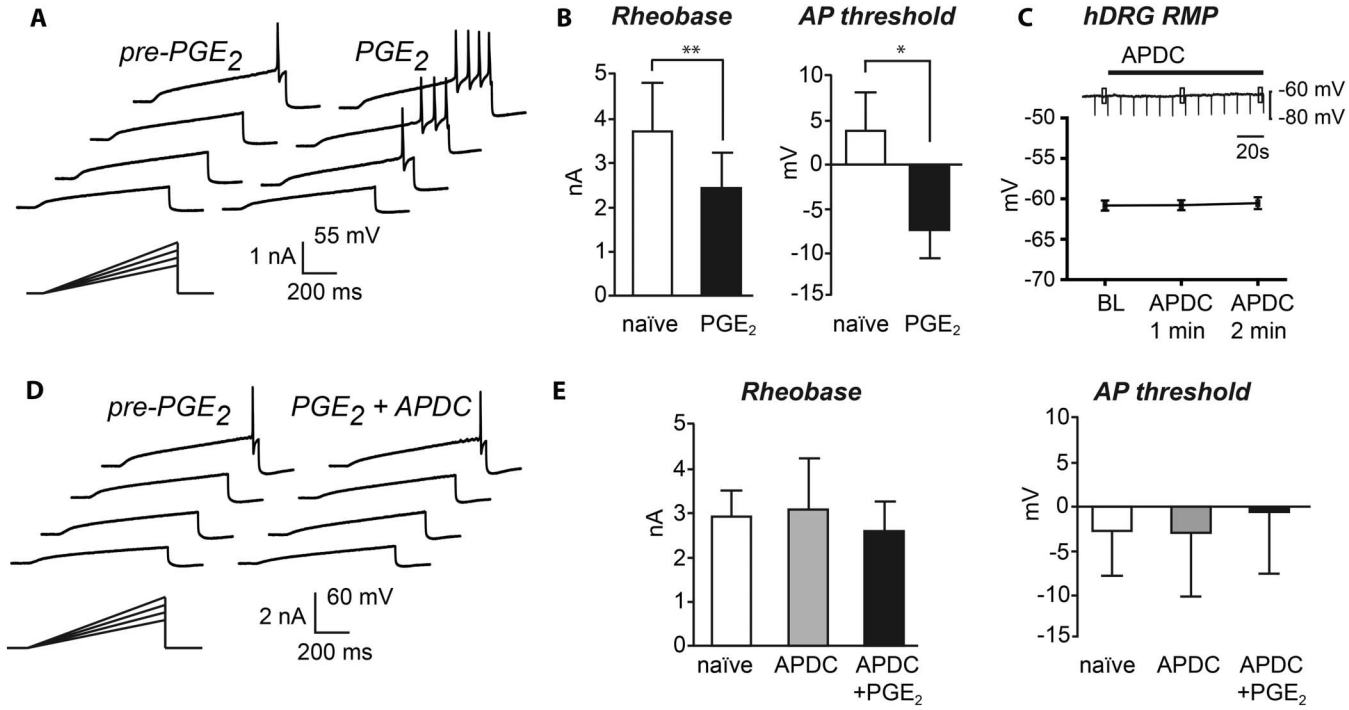

Figure 4. Block of PGE_2-induced sensitization by group II mGluRs in human sensory neurons. (A) Example of sensitization of an hDRG neuron with PGE_2. (B) PGE_2-induced sensitization led to decreased rheobase and hyperpolarized action potential threshold of hDRG neurons (paired *t* test $^{**}P < 0.01$, $^{*}P < 0.05$; n = 11). (C) Application of APDC to human sensory neurons produced no changes to the resting membrane potential or input resistance in vitro (before APDC: 383 ± 75.6 MΩ; 2 minutes after APDC: 385 ± 76.1 MΩ; $P = 0.78$; mean ± SEM, n = 10). (D) PGE_2-induced sensitization was blocked by APDC (1 μM). (E) APDC alone did not alter membrane excitability but prevented PGE_2-induced sensitization (n = 9).

related to inhibition of the heat- and capsaicin-sensitive ion channel TRPV1.[3,6] However, it was not established whether the analgesic effects of group II mGluRs were linked specifically to reducing TRPV1-evoked activity or whether a broader effect on membrane excitability exists. Indeed, although group II mGluR agonists display analgesic efficacy in thermal tests of nociceptive behavior as would be expected from a TRPV1-dependent mechanism, studies have also shown analgesic effects on neuropathic and formalin-induced pain models believed to be TRPV1 independent.[43,57] In this study, we show that APDC raised the rheobase and threshold for firing action potentials in PGE_2-exposed sensory neurons, indicating that group II mGluRs reduce overall membrane excitability under inflammatory conditions, thus supporting an alternative mechanism for group II mGluR analgesia. Further evidence for the conclusion that group II mGluRs control sensory neuron membrane excitability was observed when forskolin-induced enhancement of tetrodotoxin-sensitive sodium currents was blocked by APDC.[55]

Group II mGluR immunoreactivity was identified in previous studies mostly in small-diameter sensory neurons, many of which bound the nonpeptidergic marker IB4, and which projected centrally to outer lamina II–IV in the dorsal horn.[4,5] These experiments were unable to differentiate between the mGlu2 and mGlu3 receptor subtypes, and because *Grm3* mRNA was previously not found in rodent DRG neurons,[36] the mGlu2 receptor subtype was believed to be solely responsible for the mGluR2/3 immunoreactivity and peripherally mediated analgesia. The present results show mGlu2-only immunoreactivity in mouse primary afferent fibers, as well as in mouse and human DRG somata. We found mGlu2 expression in a heterologous population of mouse DRG neurons including small-diameter nociceptors marked with CGRP or IB4, although few cells colabeled with the latter. Immunoreactivity for mGlu2 was also detected in larger neurons in both mice and humans, raising the question of whether group II agonists contribute to analgesia in part by acting on larger neurons, many of which are likely A-beta mechanoreceptors. Mechanoreceptors are believed to contribute to both neuropathic and inflammatory pain and can undergo phenotypic switching after injury-facilitating nociceptive signal transduction.[15] Although the mechanisms of sensitization and nociception in large-diameter neurons require further study, mGlu2 could play an inhibitory role in suppressing, eg, injury-induced ectopic activity. Differences between the present results and previous studies[4,5] may be attributed to altered mGlu2 localization between the rat and mouse, the distinct patterns of IB4 staining between these species,[40] and the use of a nonselective mGlu2/3 antibody in previous work.

Through quantitative RNA-seq, we show that expression of transcripts for *GRM2* and *GRM3* are present in the DRG of both mice and humans. The abundance of *GRM3* was comparatively low in mouse DRG, perhaps explaining the lack of detection in rodent using earlier techniques, but its presence may nevertheless contribute to the suppression of PGE_2-induced hyperexcitability by APDC that we detected in physiological experiments. Interestingly, the expression levels of *GRM2* and *GRM3* transcript appeared equivalent in human DRG, introducing the idea of potentially targeting mGluR3 for analgesia in humans. Our strategy for RNA-seq used whole DRG containing a heterogeneous population of neurons, satellite glia, and possibly nonneuronal cells from vasculature or meninges near the ganglia. It is important to develop the techniques that will allow further human work to determine the precise localization of *GRM2* and *GRM3* and other transcripts at the single-neuron level.

Group II mGluR expression is epigenetically modulated, involving upregulation by NFκB transcription factors or by injury,

and blocking the degradation of these transcription factors to increase *GRM2* has been identified as a possible analgesic therapy.[8,9,37] This dynamically regulated expression also suggests that group II mGluR levels in neurons processed for culture could differ from those preserved for immunohistochemistry. Sensory neurons below the threshold for immunohistochemical identification may be capable of upregulating group II mGluRs under certain conditions allowing agonists or positive allosteric modulators a broader ability to contribute to physiological and behavioral responses.

Group II mGluRs are well positioned to regulate nociception at the first stage of sensory transduction, including within the visceral system where mGlu2 was found in the nodose ganglia and the evoked discharge of visceral afferents could be inhibited by APDC.[2,38] The antinociceptive effect seems to be limited to inflammatory or potential injury states because activation of group II mGluRs in the naive state did not alter nociceptor excitability. An analogous result was observed when nociceptor fiber discharge during a brief heat stimulus was not attenuated by APDC, but the discharge generated by the longer-lasting algogen capsaicin was reduced.[6] The release of glutamate into the skin by keratinocytes[19] or reflex activation of the peripheral terminals of primary afferent fibers[25,30] under injury or inflammatory conditions could produce endogenous activation of group II mGluRs leading to negative feedback and inhibition of the ongoing response. This schema is consistent with the presynaptic inhibitory effect of group II mGluRs reported in other parts of the nervous system,[42] with the receptive peripheral ending from these pseudounipolar axons functioning analogously to a presynaptic terminal. Group II mGluRs likely also act presynaptically at the central terminal reducing neurotransmission from primary afferents at the first synapse in the dorsal horn.[20]

Direct agonists for group II mGluRs have been efficacious for relief of pain in animal studies,[26] but have only been tested in human clinical trials for non–pain-related indications.[17,39] It remains unclear whether direct, ligand-activated group II mGluRs likewise reduces pain. Although group II mGluR agonists have not been tested for pain relief in humans, dietary supplements that modulate mGlu2 expression have shown some analgesic benefits.[10,27] A recent human study showed that N-acetyl-cysteine reduced laser-evoked pain ratings likely through a group II mGluR-dependent mechanism, while leaving thermal threshold detection unchanged.[46] In addition to direct agonists, enhanced endogenous activation by recently derived positive allosteric modulators can be used to control mGlu2 signaling.[48]

A few recent studies have begun to investigate the physiology of viable human DRG neurons and have highlighted some similarities and potentially important differences in how human DRG neurons respond to stimuli previously tested only in rodents.[14,29,31,52] However, our study is the first to show that inflammation-induced sensitization of human nociceptors, a neural correlate of hyperalgesia, can be blocked by a candidate analgesic in vitro. The present data demonstrate that a mechanism of group II mGluR-induced analgesia identified in rodent sensory neurons translates mechanistically to human sensory neurons. Group II mGluR agonists and positive allosteric modulators have already demonstrated acceptable safety profiles in clinical trials[50] making them attractive candidates for the development of a peripherally acting, activity-dependent analgesic.

Conflict of interest statement

A. Ghetti is a paid employee of AnaBios Corporation. AnaBios Corporation provided a subset of the human DRG used in these studies. All other authors declare no competing financial interests or conflicts of interest.

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