SARS-CoV-2 spike protein co-opts VEGF-A/neuropilin-1 receptor signaling to induce analgesia

Aubin Moutal, Laurent F. Martin, Lisa Boinon, Kimberly Gomez, Dongzhi Ran, Yuan Zhou, Harrison J. Stratton, Song Cai, Shizhen Luo, Kerry Beth Gonzalez, Samantha Perez-Miller, Amol Patwardhan, Mohab M. Ibrahim, Rajesh Khanna

Abstract

Global spread of severe acute respiratory syndrome coronavirus 2 continues unabated. Binding of severe acute respiratory syndrome coronavirus 2’s spike protein to host angiotensin-converting enzyme 2 triggers viral entry, but other proteins may participate, including the neuropilin-1 receptor (NRP-1). Because both spike protein and vascular endothelial growth factor-A (VEGF-A)—a pronociceptive and angiogenic factor, bind NRP-1, we tested whether spike could block VEGF-A/NRP-1 signaling. VEGF-A-triggered sensory neuron firing was blocked by spike protein and NRP-1 inhibitor EG00229. Pronociceptive behaviors of VEGF-A were similarly blocked through suppression of spontaneous spinal synaptic activity and reduction of electrogenic currents in sensory neurons. Remarkably, preventing VEGF-A/NRP-1 signaling was antiallodynic in a neuropathic pain model. A “silencing” of pain through subversion of VEGF-A/NRP-1 signaling may underlie increased disease transmission in asymptomatic individuals.

Keywords: SARS-CoV-2, Spike, neuropilin-1, VEGF-A, Ca2+, sodium channels, neuropathic pain

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of COVID-19, a coronavirus disease that, as of August 24, has infected more than 23.5 million people and caused nearly 810,000 deaths worldwide. Most patients infected with SARS-CoV-2 report mild to severe respiratory illness with symptoms such as fever, cough, and shortness of breath. However, a subset of patients diagnosed by a positive nucleic acid test are either asymptomatic or minimally symptomatic. Increasing evidence shows that asymptomatic individuals can spread the virus efficiently, and the emergence of these silent spreaders of SARS-CoV-2 has limited control of the pandemic. Pain is a rising concern in symptomatic patients, likely emanating from a direct attack of SARS-CoV-2 on cells and the “cytokine storm” unleashed by affected cells. Whether asymptomatic or minimally symptomatic individuals have reduced pain thresholds, or whether their pain is silenced is unknown, but either could contribute to increased disease transmission dynamics.

The surface expressed angiotensin-converting enzyme 2 (ACE2) has been lionized as the main receptor for uptake of SARS-CoV-2. Emerging evidence points to a subset of ACE2-expressing sensory neurons that synapse with spinal and brainstem CNS neurons to produce neurological effects, including headache and nerve pain. Curiously, ACE2 is not present in most neurons, despite increasing reports of neurological symptoms being common in COVID-19 patients. Paradoxically, although the levels of ACE2 expression decline with age, increased COVID-19 severity was noted in older patient populations, such as that of Italy’s, supporting the contention that ACE2 is not the sole gateway for entry of SARS-CoV-2.

Two recent reports demonstrated that the SARS-CoV-2 spike protein can bind to the b1b2 domain of the neuropilin-1 receptor (NRP-1). This interaction occurs through a polybasic amino acid sequence (ISGERLRAR), not conserved in SARS and Middle East Respiratory Syndrome (MERS), termed the “C-end rule” (CendR) motif, which significantly potentiates its entry into cells. Importantly, “omic” analyses revealed a significant upregulation of NRP-1 in biological samples from COVID-19 patients compared to healthy controls. Using vascular endothelial growth factor-A (VEGF-A), a physiological ligand for the b1b2 pocket in NRP-1, we interrogated whether the spike protein, the major surface antigen of SARS-CoV-2, could block VEGF-A/NRP-1 signaling to affect pain behaviors. Given parallels between the pronociceptive effects of VEGF-A in rodents and humans and clinical findings demonstrating increased VEGF-A levels in bronchial alveolar lavage fluid from COVID-19...
patients coupled with substantially lower levels in the sera of asymptomatic individuals compared to symptomatic patients, a secondary question was to test whether spike protein could confer analgesia. We found that VEGF-A sensitizes nociceptor activity—a hallmark of neuropathic pain, which was blocked by the spike protein and NRP-1 inhibitor EG00229. Furthermore, we identify a novel analgesic role for spike protein, which is mirrored by NRP-1 inhibition.

2. Materials and methods

An expanded version of all Methods is available in the Supplementary Information section (available online at http://links.lww.com/PAIN/B190).

2.1. Animals

Pathogen-free adult male and female Sprague-Dawley rats (225-250 g; Envigo, Indianapolis, IN) were housed in temperature-controlled (23 ± 3°C) and light-controlled (12-hour light/12-hour dark cycle; lights on 07:00-19:00) rooms with standard rodent chow and water available ad libitum. The Institutional Animal Care and Use Committee of the College of Medicine at the University of Arizona approved all experiments. All procedures were conducted in accordance with the Guide for Care and Use of Laboratory Animals published by the National Institutes of Health. Animals were randomly assigned to treatment or control groups for the behavioral experiments. Animals were initially housed 3 per cage but individually housed after the intrathecal cannulation. All behavioral experiments were performed by experimenters who were blinded to the experimental groups and treatments.

2.2. Enzyme-linked immunosorbent assay-based NRP1–spike protein binding assay

Plates (96-well, Nunc Maxisorp; Thermo Fisher Scientific, Waltham, MA) were coated with human Neurofilin-1-Fc (10 ng per well, Cat# 50-101-8343, Fisher, Hampton, NH) and incubated at room temperature overnight. The following day, the plates were washed and blocked with 3% bovine serum albumin (BSA) in phosphate buffered saline (PBS) to minimize nonspecific adsorptive binding to the plates. SARS-CoV2 spike protein (S1 domain aa16-685, Cat# Z03485; Genscript, Piscatway, NJ) was added at concentrations ranging from 500 to 0.07 nM. As a negative control, some wells received PBS containing 3% BSA. The plates were incubated at room temperature with shaking for 3 hours. Next, the plates were washed with PBS to eliminate unbound protein. Bound SARS-CoV-2 Spike was detected by anti-His probe HRP (Cat#15165; Thermo Fisher Scientific). Tetramethylbenzidine (Cat#DY999; R&D Systems, St. Louis, MO) was used as the colorimetric substrate. The optical density of each well was determined immediately, using a microplate reader (Multiskan Ascent; Thermo Fisher Scientific) set to 450 nm with a correction wavelength of 570 nm. Data were analysed by nonlinear regression analysis using GraphPad Prtism 8 (GraphPad, San Diego, CA).

2.3. Preparation of acutely dissociated dorsal root ganglion neurons

Dorsal root ganglia (DRG) from all levels were acutely dissociated using methods as described previously. Rat DRG neurons were isolated from 100 g female Sprague-Dawley rats using previously developed procedures.

2.4. Culturing primary dorsal root ganglia neurons and microelectrode array analysis

Dissociated DRG neurons were maintained in media containing Neurobasal (Cat# 21103049; Thermo Fisher), 2% B-27 (Cat# 17504044; Thermo Fisher), 1% penicillin/streptomycin sulfate from 10,000 µg/mL stock, 30 ng/mL nerve growth factor, and 10% fetal bovine serum (HyClone R&D Systems, Minneapolis, MN). Collected cells were resuspended in DRG media and seeded as a 10 µl drop on the poly-D-lysine coated electrodes of the microelectrode array (MEA) (24-well plate, Cat# MED-Q2430L, Alpha Med Scientific, Osaka, Japan). The cells were allowed to adhere for 30 minutes and then flooded with DRG media. The next day, cells were analyzed on a MED64 presto where 24 wells, with each containing 16 electrodes, could be recorded simultaneously. Cells were treated with the indicated neuropilin 1 ligands Semaphorin 3A (3 nM; Cat#5926-S3-025; R&D Systems), VEGF-A165 (1 nM, Cat#P4853; Abnova), or VEGF-B (1 nM, Cat#RPU44324; Biomatik) for 30 minutes before recording. Treatments with either spike (100 nM, Cat#Z03485; Genscript) or the NRP-1 inhibitor EG-00229 (30 µM, Cat#6986; Tocris Bioscience) were conducted 30 minutes before adding VEGF-A. The data were analyzed on MEA symphony and Mobius offline toolkit to extract the firing rate of the active electrodes before and after treatment. Firing rate is shown as Hz (event per second) for the electrodes that showed spontaneous activity.

2.5. Whole-cell patch recordings of Na⁺ and N-type (Ca₂⁺) currents in acutely dissociated dorsal root ganglia neurons

Recordings were obtained from acutely dissociated DRG neurons as described previously. The solutions and protocols for measuring sodium currents were exactly as described. The protocol for isolating N-type calcium currents was previously described by Khanna et al. To isolate the N-type channel in DRG, contributions of the high- and low-voltage-activated calcium channel subtypes were blocked with the following subunit-selective blockers (all purchased from Alomone Labs, Jerusalem): Nifedipine (10 µM, L-type); ω-agatoxin GIVA (200 nM, P/Q-type); SNX-482 (200 nM, R-type); and TTA-P2 (1 µM, T-type). Dorsal root ganglia neurons were interrogated with current–voltage (I-V) and activation/inactivation voltage protocols as previously described.

To determine the effect of VEGF-A application on voltage-gated sodium and calcium currents, we incubated recombinant rat VEGF-A (1 nM in PBS) with DRG neurons for 30 minutes before whole-cell recordings. In addition, recombinant spike protein (100 nM in PBS) and the neuropilin 1 (NRP-1) blocker EG00229 (30 µM in DMSO, Cat. No. 6986; Tocris Bioscience) were also applied to the culture medium for 30 minutes before recording. For experiments where the proteins were tested in combination, the spike protein was added first for 30 minutes, followed by VEGF-A and EG00229 before recording commenced. The control conditions used either PBS, dimethyl sulfoxide (DMSO), or both to match the solutions used in the experimental conditions. The proteins and blocker were included at the same concentrations in the extracellular recording solution during all data acquisition.

2.6. Preparation of spinal cord slices

As described before, transverse 380-µm thick transverse slices were prepared from young rats (postnatal 10-14 days) for electrophysiological recordings at room temperature. VEGFA (1 nM), NRP-1 inhibitor (EG00229, 30 µM), and spike protein were...
added directly to the recording solution as indicated. Slices were treated for 30 minutes before the recordings.

2.7. Electrophysiological recording in spinal cord slices by whole-cell patch clamp
Substantia gelatinosa neurons were recorded using previously described methods.55

2.8. Hind paw injection procedures
PBS vehicle (NaCl 137 mM, KCl 2.5 mM, Na₂HPO₄ 10 mM, and KH₂PO₄ 1.8 mM), VEGF-A₁₆₅ (10 nM), spike (100 nM), and EG00229 (30 μM) were injected subcutaneously, alone or in combination, in the dorsum of the left hind paw. Rats were gently restrained under a fabric cloth, and 50 μL was injected using 0.5-mL syringes (27-G needles).

2.9. Implantation of intrathecal catheter
For intrathecal (i.t.) drug administration, rats were chronically implanted with catheters as described by Yaksh and Rudy.63

2.10. Testing of allodynia
The assessment of tactile allodynia (ie, a decreased threshold to paw withdrawal after probing with normally innocuous mechanical stimuli) using a series of calibrated fine (von Frey) filaments was performed as described in Chaplan et al.8

2.11. Spared nerve injury
Nerve injury was inflicted as described previously.12 Rats were tested on day 10 after the surgery, at which time they had developed stable and maximal mechanical allodynia.

2.12. Synapse enrichment and fractionation
Adult rats were anesthetized using isoflurane and decapitated. Spinal cords were removed, and the dorsal horn of the spinal cord was dissected because this structure contains the synapses arising from the DRG. Synaptosome isolation was performed as described in Chaplan et al.8

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2.13. Immunoblot preparation and analysis
Protein concentrations were determined using the BCA protein assay (Cat# P23225; Thermo Fisher Scientific). Indicated samples were loaded on 4% to 20% Novex gels (Cat# EC60285BOX; Thermo Fisher Scientific). Proteins were transferred for 1 hour at 120 V using TBS (25 mM Tris pH = 8.5, 192 mM glycerine, 0.1% (mass/vol) SDS), 20% (vol/vol) methanol as transfer buffer to polyvinylidene difluoride membranes 0.45 μm (Cat # IPVH00010; Millipore, Billerica, MA), preactivated in pure methanol. After transfer, the membranes were blocked at room temperature for 1 hour with TBST (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.1% Tween 20), 5% (mass/vol) nonfat dry milk, then incubated separately in the primary antibodies VEGFR2 (Cat#PA5-16487; Thermo Fisher), pY1175 VEGFR2 (Cat#PA5-105167; Thermo Fisher), Flotillin (Cat#F1180; Sigma-Aldrich, St Louis, MO), or neurelin-1 (Cat#sc-5307; Santa Cruz Biotechnology, Dallas, TX) in TBST, 5% (mass/vol) BSA, overnight at 4°C. After incubation in horseradish peroxidase-conjugated secondary antibodies from Jackson ImmunoResearch, blots were revealed by enhanced luminescence (WBKLS0500; Millipore) before exposure to photographic film. Films were scanned, digitized, and quantified using Un-Scan-It gel version 7.1 scanning software by Silk Scientific Inc.

2.14. Docking of vascular endothelial growth factor-A spike protein and EG00229 to NRP-1
Peptide from C-terminus of furin cleaved SARS-CoV-2 spike protein 681-PRRAR-685 (blue sticks) was docked to NRP-1-b1 domain (white surface with binding site in red; PDB 6fmc46). The extracellular portion of NRP-1 was confirmed by enzyme-linked immunosorbent assay (Fig. 1A). We calculated an equilibrium constant of dissociation (Kd) for this interaction to be ~166.2 nM (Fig. 1A). We next plated sensory neurons on multwell MEAs, an approach enabling multiplexed measurement of spontaneous, as well as stimulus-evoked extracellular action potentials from large populations of cells.9 Vascular endothelial growth factor-A increased spontaneous firing of DRG neurons, which was blocked by the S1 domain of the spike protein and by the NRP-1 inhibitor EG00229 (Fig. 1A). In contrast, ligands VEGF-B (ligand for VEGFR1—a co-receptor for NRP-131) and semaphorin 3A (Sema3A, ligand for plexin receptor—also a co-receptor for NRP-113,53) did not affect the spontaneous firing of nociceptors (Figs. 1B and C). The lack of effect of VEGF-B and Sema3A rules out a role for VEGF-R1 and plexin, respectively, thus implicating a novel ligand-, VEGF-A, and receptor-, NRP-1, specific pathway driving nociceptor firing (Fig. 1D).

Because both VEGF-A and spike protein share a common binding pocket on NRP-1 (Fig. 1C),10,11,43 we asked if the spike protein could block VEGF-A/NRP-1 signaling to affect pain behaviors. Consistent with previous reports,4,58 we confirmed that VEGF-A is pronociceptive as intraplantar injection of VEGF-A decreased both paw withdrawal thresholds (PWTs) (Figs. 2A and 3.1. Ligand specific engagement of NRP-1 signaling induces nociceptor activity and pain
Initially, we assessed the involvement of spike and NRP-1 in the VEGF-A/NRP-1 pathway. An interaction between spike (S1 domain aa 16-685, containing the CendR motif 682RRAR685) and the extracellular portion of NRP-1 was confirmed by enzyme-linked immunosorbent assay (Fig. 1A). We calculated an equilibrium constant of dissociation (Kd) for this interaction to be ~166.2 nM (Fig. 1A). Next, we plated sensory neurons on multwell MEAs, an approach enabling multiplexed measurement of spontaneous, as well as stimulus-evoked extracellular action potentials from large populations of cells.9 Vascular endothelial growth factor-A increased spontaneous firing of DRG neurons, which was blocked by the S1 domain of the spike protein and by the NRP-1 inhibitor EG00229 (Fig. 1A). In contrast, ligands VEGF-B (ligand for VEGFR1—a co-receptor for NRP-131) and semaphorin 3A (Sema3A, ligand for plexin receptor—also a co-receptor for NRP-113,53) did not affect the spontaneous firing of nociceptors (Figs. 1B and C). The lack of effect of VEGF-B and Sema3A rules out a role for VEGF-R1 and plexin, respectively, thus implicating a novel ligand-, VEGF-A, and receptor-, NRP-1, specific pathway driving nociceptor firing (Fig. 1D).

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B and Table S1, http://links.lww.com/PAIN/B190) and latencies to a thermal stimulus (Figs. 2C and D and Table S1, http://links.lww.com/PAIN/B190) in male rats. Similar results were obtained in female rats as well (Figs 2E–H and Table S1, http://links.lww.com/PAIN/B190). Preventing VEGF-A from binding to NRP-1 with the NRP-1 inhibitor EG00229 or spike from activating VEGF-A/NRP-1 signaling blunted the mechanical allodynia and thermal hyperalgesia induced by VEGF-A alone (Fig. 2 and Table S1, http://links.lww.com/PAIN/B190). Neither Spike nor EG00229 alone had any effect on these behaviors (Fig. 2 and Table S1, http://links.lww.com/PAIN/B190) in either sex. Together, these data provide functional evidence that VEGF-A/NRP-1 signaling promotes a pain-like phenotype by sensitizing nociceptor activity (Fig. 1D).

3.2. Vascular endothelial growth factor–mediated increases in dorsal root ganglia ion channel currents are normalized by disruption of VEGF-A/NRP-1 signaling

To gain insight into the mechanism by which VEGF-A contributed to increased nociceptor activity, we postulated that ion channels in DRG may be affected because these contribute to nociceptive plasticity.61 Typical families of Na\(^+\) currents from small-diameter DRG neurons are shown in Figure 3A. Vascular endothelial growth factor-A facilitated a 1.9-fold increase in total Na\(^+\) currents compared to vehicle (PBS)-treated DRG, which was completely blocked by spike protein (Figs. 3B and C). Spike protein alone did not affect Na\(^+\) currents (Figs. 3B and C and Table S1, http://links.lww.com/PAIN/B190). Because this decreased current could arise from changes in channel gating, we determined whether activation and inactivation kinetics of DRG Na\(^+\) currents were affected. Half-maximal activation and inactivation (V\(_{1/2}\)), as well as slope values (k) for activation and inactivation, were not different between the conditions tested (Figs. 3D and E and Tables S1, S2, http://links.lww.com/PAIN/B190), except for an ~8-mV hyperpolarizing shift in sodium channel inactivation induced by cotreatment of VEGF-A and EG00229 (Table S2, http://links.lww.com/PAIN/B190). Similar results were obtained for the NRP-1 inhibitor EG00229, which also inhibited the VEGF-A-mediated increase in total Na\(^+\) currents (Figs. 3F–H and Table S1, http://links.lww.com/PAIN/B190) but had no effect on the biophysical properties (Figs. 3I and J and Tables S1, S2, http://links.lww.com/PAIN/B190).

Because calcium channels play multiple critical roles in the transmission and processing of pain-related information within...
the primary afferent pain pathway, we evaluated whether they were affected. We focused on N-type (Ca\textsuperscript{v2.2}) channels because these mediate neurotransmitter release at afferent fiber synapses in the dorsal horn and are critical in the pain matrix. Vascular endothelial growth factor-A facilitated a 1.8-fold increase in total Ca\textsuperscript{2+} currents compared to vehicle (PBS)-treated DRG, which was completely blocked by spike protein (Figs. 4A–C and Table S1, http://links.lww.com/PAIN/B190). Spike protein alone did not affect Ca\textsuperscript{2+} currents (Figs. 4A–C).

In addition, we did not observe any changes in activation and inactivation kinetics between the conditions tested (Figs. 4D and E and Tables S1 and S2, http://links.lww.com/PAIN/B190). Similar results were obtained for the NRP-1 inhibitor EG00229, which inhibited the VEGF-A-mediated increase in N-type Ca\textsuperscript{2+} currents (Figs. 4F–H and Table S1, http://links.lww.com/PAIN/B190) but had no effect on the biophysical properties (Figs. 4I and J and Tables S1 and S2, http://links.lww.com/PAIN/B190). These data implicate spike protein and NRP-1 in Na\textsuperscript{+} and Ca\textsuperscript{2+} (Ca\textsuperscript{v2.2}) channels in VEGF-A/NRP-1 signaling.

3.3. Vascular endothelial growth factor-A enhances synaptic activity in the lumbar dorsal horn that is normalized by inhibition of NRP-1 signaling and spike protein

The spinal cord is an integrator of sensory transmission where incoming nociceptive signals undergo convergence and modulation. Spinal presynaptic neurotransmission relies on DRG neuron action potential firing and neurotransmitter release. From these fundamental physiological principles, as well as the results described above, we were prompted to evaluate whether synaptic activity was affected in the lumbar dorsal horn. The amplitude of spontaneous excitatory postsynaptic currents (sEPSCs) of neurons in the substantia gelatinosa region of the lumbar dorsal horn were not affected by VEGF-A (Figs. 5A and B and Table S1, http://links.lww.com/PAIN/B190). In contrast, VEGF-A application increased sEPSC frequency by 3.6-fold, which was reduced by 57% by inhibition of NRP-1 with EG00229 and 50% by spike protein (Figs. 5A and C and Table S1, http://links.lww.com/PAIN/B190). Amplitude and interevent interval cumulative distribution curves for sEPSCs are shown in Figures 5D and E. When compared to vehicle controls, VEGF-A, with or without NRP-1 inhibitor or spike protein, had no effect on the cumulative amplitude distribution of the spontaneous EPSCs (Fig. 5D and Table S1, http://links.lww.com/PAIN/B190) but changed the cumulative frequency distribution of spontaneous EPSCs with significantly longer interevent intervals (Fig. 5E and Table S1, http://links.lww.com/PAIN/B190). Together, these data suggest a presynaptic mechanism of action for spike protein and NRP-1.

3.4. Spike protein and inhibition of NRP-1 confer antinociception in the spared nerve injury model of chronic neuropathic pain

We used the spared nerve injury (SNI) model of neuropathic pain, chosen because it produces a reliable and consistent increase in pain sensitivity, to evaluate potential disruption of the VEGF-A/NRP-1 pathway to reverse nociception. Vascular endothelial growth factor-A triggers autophosphorylation of VEGFR2 at Y1175, thereby serving as a proxy for activation of VEGF-A signaling. In rats with SNI, intrathecal application of spike decreased the phosphorylation of VEGFR2 (Y1175) on both the contralateral (noninjured) and ipsilateral (injured) sides (Figs. 6A and B). This shows that spike can inhibit VEGF-A signaling in a rat model of chronic neuropathic pain. Spared nerve injury
efficiently reduced PWTs (mechanical allodynia, Fig. 6C and Table S1, http://links.lww.com/PAIN/B190) 10 days after injury. Spinal administration of spike protein significantly increased PWTs (Fig. 6C and Table S1, http://links.lww.com/PAIN/B190), in a dose-dependent manner, for 5 hours. Analysis of the area under the curve confirmed the dose-dependent reversal of mechanical allodynia (Fig. 6D and Table S1, http://links.lww.com/PAIN/B190) compared to vehicle-treated injured animals. Similar results were seen with female rats injected with spike (Figs. 6E and F). Finally, inhibition of NRP-1 signaling with EG00229 also reversed paw withdrawal thresholds (Figs. 6G and H and Table S1, http://links.lww.com/PAIN/B190).

4. Discussion
Our data show that SARS-CoV-2 spike protein subverts VEGF-A/NRP-1 pronociceptive signaling. Relevant to chronic neuropathic pain, spike negated VEGF-A-mediated increases in: (1) voltage-gated sodium and N-type calcium current densities in DRG neurons; (2) spontaneous firing in DRG neurons; (3) spinal neurotransmission; and (4) mechanical allodynia and thermal hyperalgesia. Consequently, spike protein was analgesic in a nerve injury rat model. Based on the reported increase in VEGF-A levels in COVID-19 patients, one would expect to observe increased pain-related symptoms. However, our data suggest that the SARS-CoV-2...
spike protein hijacks NRP-1 signaling to ameliorate VEGF-A-mediated pain. This raises the possibility that pain, as an early symptom of COVID-19, may be directly dampened by the SARS-CoV-2 spike protein. Our results do not exclude the possibility that alternative fragments of spike or other viral proteins may be pronociceptive. Leveraging this atypical function of SARS-CoV-2 spike protein may yield a novel class of therapeutics for pain.

Clinical findings that VEGF-A contributes to pain are supported by observations that in osteoarthritis, increased VEGF expression in synovial fluids has been associated with higher pain scores. Vascular endothelial growth factor-A has been reported to enhance pain behaviors in normal, nerve-injured, and diabetic animals. Blocking VEGF-A has been shown to reduce nociception in rodents and to exert a neuroprotective effect by improving neuronal restoration and conduction, decreasing proapoptotic Caspase-3 levels in sensory neurons, preventing neural perfusion and epidermal sensory fiber loss. Alternative splicing of the VEGF-A gene produces several isoforms of the mature protein containing between 121 and 206 amino acid residues, with VEGF-A165a being pronociceptive through sensitization of transient receptor potential

Figure 5. Spontaneous excitatory postsynaptic current frequency is reduced by spike protein or pharmacological antagonism of neuropilin-1 receptor (NRP-1) by EG00229. (A) Representative traces of sEPSC from neurons of the substantia gelatinosa in the superficial dorsal horn (lamina I/II) treated for at least 30 minutes with the indicated conditions. Summary of amplitudes (B) and frequencies (C) of sEPSCs for all groups is shown. Cumulative distribution of the sEPSC amplitude (D) and the interevent interval (E) recorded from cells as indicated. Perfusion of 30 μM EG00229 decreased spontaneous excitatory synaptic transmission (A–E) in lumbar dorsal horn neurons. P values of comparisons between treatments are as indicated; for full statistical analyses, see Table S1, http://links.lww.com/PAIN/B190. sEPSC, spontaneous excitatory postsynaptic current.
channels^2 and ATP-gated purinergic P2X2/3 receptors^27 in DRG neurons. VEGF-A165b is a VEGF receptor-2 partial agonist that competes with VEGF-A165a for binding to VEGF-R2. The VEGF-A165a isoform also binds to the NRP-1 coreceptor, whereas VEGF-A165b does not. Thus, it is a balance of the splice isoforms (VEGF-Aa/b) that likely determines the nociceptive impact on sensory neurons.

Our data show that VEGF-A elicits long-lasting (up to 24 hours) mechanical allodynia and thermal hyperalgesia in male and female naive rats, thus supporting the premise that VEGF-A is pronociceptive. Vascular endothelial growth factor is augmented in serum of rheumatoid arthritis patients.^

In chronic neuropathic pain, a concomitant increase of NRP-1 and VEGF-A has been reported in DRG neurons.^

Altogether, our data suggest that interfering with VEGF-A-NRP-1 using SARS-CoV-2 spike or the NRP-1 inhibitor EG00229 signaling in pain. Our working model shows that VEGF-A engagement of NRP-1 is blocked by spike protein, consequently decreasing activities of two key nociceptive voltage-gated sodium, likely Nav1.7, and calcium (Cav2.2) channels (Fig. 1D). The resulting decrease in spontaneous DRG neuronal firing by spike protein translates into a reduction in pain (Fig. 1D).

Characterization of the molecular cascade downstream of VEGF-A/NRP-1 signaling awaits further work.

Conflict of interest statement

R. Khanna is the cofounder of Regulonix LLC, a company developing nonopioid drugs for chronic pain. In addition,
R. Khanna has patents US10287334 and US10441586 issued to Regulonix LLC. R. The remaining authors have no conflicts of interest.

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Author contributions: R.K. and A.M. developed the concept and designed experiments; A.M., L.F.M., L.B., K.G., D.R., Y.Z., H.J.S., S.C. performed electrophysiology recordings; S.P.-M. assisted with docking studies; A.P. and M.M.I. provided funding for L.F.M.; R.K. and A.M. wrote the manuscript; and R.K. and A.M. supervised all aspects of this project. All authors had the opportunity to discuss results and comment on the manuscript. Data and materials availability: All data are available in the main text, figures, and supplementary materials (available online at http://links.lww.com/PAIN/B190).

Appendix A. Supplemental digital content

Supplemental digital content associated with this article can be found online at http://links.lww.com/PAIN/B190.

Supplemental video content

A video abstract associated with this article can be found at http://links.lww.com/PAIN/B191.

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