hypothesize that CGRP exerts a dual effect on both cancer-associated pain and tumor progression, suggesting that CGRP may be a promising therapeutic target in HNSCC treatment. We used human tumor tissue and patient-reported outcomes to explore the relationship between CGRP+ sensory nerve innervation and cancer pain in patients. To determine CGRP receptor expression on tumor cells, immunohistochemistry and PCR were performed on human and mouse oral cancer cell lines. We used a syngeneic tongue tumor transplant mouse model of oral cancer and a global Calca knockout mouse (i.e. CGRP-KO) to investigate the impact of CGRP signaling on tumor growth and the associate immune response in vivo. We found prominent CGRP-immunoreactive sensory nerve presence innervating human HNSCC tumor tissue, which positively correlated to patient-reported pain (r2=0.357). Furthermore, human HNSCC cell lines expressed 3-fold more CGRP receptor, RAMP1, compared to a non-tumorigenic keratinocyte cell line. In tumor-bearing CGRP-KO mice, we found a significant reduction in tumor size at post-inoculation days 7 and 14 compared to wildtype. We also found a 4-fold increase in tumor infiltrating RAMP1-expressing CD4+ T cells, as well as a 5-fold increase cytotoxic CD8+ T cells and NK1.1+ NK cells in tumor tissue CGRP-KO mice compared to wildtype. This preliminary data suggests that CGRP signaling from sensory neurons may increase cancer associated pain and tumor progression. Further knowledge regarding the relationship between sensory neurons and cancer could allow for the repurposing clinically available nervous system drugs (e.g., anti-CGRP antibodies) for the treatment of cancer and cancer pain. Grant support from the Rita Allen Foundation.

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Expression and Treatment of Pain-Depressed Climbing in Male and Female Mice

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This study evaluated climbing behavior by mice as a potential endpoint for preclinical studies on expression and treatment pain-depressed behavior. Climbing by adult male and female ICR mice was evaluated in plexiglass cylinders (11.25 cm diameter x 25.5 cm height) equipped with 0.5 cm2 wire mesh covering the inner walls. Climbing was quantified as total seconds with all four paws off the cylinder floor and at least one paw on the mesh during each 10-min behavioral session. A sequence of four experiments was conducted in separate groups of at least 12 mice (6 male, 6 female) for each experiment. Experiment 1 evaluated stability of climbing in the absence of any treatment over five test sessions conducted on Tuesdays and Fridays over a 2-week period. Experiment 2 evaluated depression of climbing by an acute, visceral noxious stimulus [intraperitoneal lactic acid (IP acid), 0-0.56% in sterile water, 10-min pretreatment time]. Experiments 3 and 4 compared effectiveness of a positive-control analgesic (the cyclooxygenase inhibitor ketoprofen, 10 mg/kg) and a negative-control non-analgesic (the centrally acting kappa opioid receptor agonist U69593, 0.1-1.0 mg/kg) to block 0.32% IP acid-induced climbing depression. For Experiments 2-4, treatments were counterbalanced across mice using a Latin-square design. Data were analyzed by one- or two-way ANOVA, and a significant ANOVA was followed by a Dunnett or Holm-Sidak post hoc test. The criterion for significance was $p<0.05$. Climbing was stable during within-subject, repeated testing and averaged 262 ± 21 sec during each 10-min session (Experiment 1). IP acid produced a concentration-dependent decrease in climbing (Experiment 2) that was blocked by ketoprofen (Experiment 3) but not by U69593 (Experiment 4). These results support utility of climbing by mice as a behavioral endpoint for studies of pain-depressed behavior and for evaluation of candidate analgesics. Grant support from NIH P30DA033934, NIH R25GM090084, and NIH T32DA007027.

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TRPV1-Expressing Sensory Neuron Stimulation-based Model of Inflammatory Injury Enhances the Excitability of Spinal Neurons Targeting the Periaqueductal Gray

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Modeling inflammatory injury via high-intensity stimulation of peripheral afferents at low frequencies (LFS) potentiates excitatory input onto spinal neurons projecting (PNs) to the midbrain periaqueductal gray (PAG), a region critical to pain processing. Therefore, we sought to identify the underlying afferent population driving this potentiation and how this enhanced synaptic drive might affect spino-PAG PN output to supraspinal pain circuits. We used 3–6-week-old male and female mice expressing Cre recombinase driven by expression of the TRPV1 gene, which is expressed in the lineage of virtually all C fibers, and Cre-dependent channelrhodopsin to stimulate C fibers. Spinal PNs were retrogradely labeled from the PAG using dil. We performed whole-cell patch-clamp recordings in spino-PAG PNs using a semi-intact spinal cord preparation. Stimulation of TRPV1-expressing (TRPV1+) peripheral afferents induced burst firing in virtually all spino-PAG PNs sampled. Inflammation increases the firing rates of C fibers to 1-2 Hz; therefore, we used LFS (1 ms, 470 nm LED pulses at 2 Hz for 2 mins) to model inflammatory injury. LFS induced a persistent ($\geq$20 min) increase in the number of action potentials (APs) within a C fiber-induced burst (n=10, p=0.029), rise in the frequency of synaptically evoked after discharge (n=10, p=0.0004), and decrease in intraburst afterhyperpolarization (n=10, p=0.037). Furthermore, LFS induced a transient membrane depolarization (Mean=6.796 mV, n=12, $p<0.0001$). Additional experiments suggested the enhanced synaptic excitability relied on postsynaptic G protein-coupled signaling, NMDA receptors, and TRPV1+ afferent input. Finally, LFS may increase the intrinsic excitability of spino-PAG PNs—by decreasing AP threshold (n=14, p=0.015) and increasing membrane resistance (n=14, p=0.0003). LFS had no effect on the membrane potential nor the firing frequency of spino-PAG PNs. In summary, this work suggests that LFS of TRPV1+ fibers persistently enhances spino-PAG projection neuron output, likely leading to a lasting increase in the activation of PAG neural circuits. Grant support from F32NS123008 NINDS; Brewer, C. B. T32DA035165 NIDA; Mackey, S. C. R01DA011289 NIDA; Kauer, J. A.

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A Novel PPAR Gamma Agonist ELB00824 Suppresses Oxaliplatin-induced Pain, Neuronal Hypersensitivity, and Oxidative Stress

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Chemotherapy-induced neuropathic pain (CINP) is a debilitating and difficult-to-treat side effect of chemotherapeutic drugs (e.g., oxaliplatin). CINP is marked with oxidative stress and neuronal hypersensitivities. The peroxisome proliferator-activated receptor gamma (PPARϒ) is a transcription factor that regulates genes involved in oxidative stress and inflammation. We hypothesize that PPARϒ agonists are protective against CIPN by reducing oxidative stress and inhibiting neuronal hypersensitivities. To test our hypothesis, we used a novel BBB (blood-brain barrier) penetrable PPARϒ agonist ELB00824 we characterized previously and a low BBB permeable PPARϒ agonist pioglitazone. CIPN was introduced in BALB/c mice (8-12 week old female) with two established treatment schedules of oxaliplatin injection (a short-lasting model with daily intraperitoneal (IP, 3 mg/kg) injection for 5 consecutive days and a long-lasting model with intravenous (IV, 5 mg/kg) injection twice per week for 4 weeks). 3, 10, or 30 mg/kg ELB00824 or pioglitazone was IP injected 5 min before each oxaliplatin treatment, and 30% DMSO, 15% Cremophor@EL in PBS buffer was used as a vehicle control. Cold allodynia, mechanical allodynia, motor coordination, sedation and addiction were measured with dry ice, von Frey filaments, beam-walking tests, and conditioned place preference, respectively. Oxidative stress was accessed by measuring spinal carbonyl groups, thiobarbituric acid reactive substance (TBARS), and nitrotyrosine levels. Neuronal hypersensitivities were measured using whole-cell current clamp recordings in isolated dorsal root ganglion neurons. In both models ELB00824, but not pioglitazone, reduced oxaliplatin-induced cold and mechanical allodynia and spinal oxidative stress. ELB0824 suppressed oxaliplatin-induced firing in IB4- neurons. ELB00824 did not cause motor discoordination or sedation/addiction or reduce the antineoplastic activity of oxaliplatin (measured with an MTS-based cell proliferation assay) in a human colon cancer cell line (HCT116) and a human oral cancer cell line (HSC-3). Our results demonstrated that ELB00824 prevents oxaliplatin-induced pain, likely via inhibiting neuronal hypersensitivities and oxidative stress. Grant support from NIH grants R01DE029493 (Y. Ye).