The pain of PIP₂

By Amy Donner, Senior Editor

A team from **The University of North Carolina at Chapel Hill** has shown that phosphatidylinositol-4-phosphate 5-kinase type 1 γ regulates signaling by diverse pain receptors and has identified a small molecule antagonist that targets it.¹ Although the compound alleviates chronic pain in multiple mouse models, the lethality of homozygous mutations of the kinase in mice and humans highlights the need for repeat-dosing studies to better characterize the safety of the approach.

Pain is triggered by the action of specialized sensory neurons known as nociceptors, which are found in skin, muscle and other tissues and are activated by noxious stimuli, including excess heat, chemical stress or physical stress.² Nociceptors detect these inputs by expressing proteins that transmit a signal from the site of the insult to the CNS, causing pain. For example, the nociceptor-expressed ion channel TRPV1 (transient receptor potential vanilloid 1; VR1) is activated by temperature or chemical stresses, triggering a signaling cascade that leads to the sensation of pain.

Although distinct receptors are responsible for detecting different stimuli, a common feature of nociception is that inflammation or injury can sensitize cells to these inputs. Indeed, most non-opioid drugs that are approved to treat pain rely on blocking inflammatory signaling rather than acting on any individual receptor.

A UNC team led by Mark Zylka and Jian Jin set out to identify and target additional pathways that could broadly modulate the activity of pain receptors.

Zylka told *SciBX*, “Tissue injury causes the release of diverse molecules that activate receptors on sensory neurons and cause pain. Given this molecular diversity, efforts to treat pain by blocking individual receptors have failed.”

Zylka is an associate professor of cell biology and physiology at UNC, and Jin is associate director of medicinal chemistry in the Center for Integrative Chemical Biology and Drug Discovery and an associate professor of chemical biology, medicinal chemistry and pharmacology at **The University of North Carolina at Chapel Hill Eshelman School of Pharmacy**.

Zylka’s team zeroed in on the synthesis of phosphatidylinositol 4,5-bisphosphate (PIP₂), a lipid that had previously been shown to regulate signaling by pain receptors. PIP₂ is generated in part by the activity of lipid kinases, but the identity of the kinase or kinases responsible for PIP₂’s synthesis in neurons of the dorsal root ganglia (DRG), in which pain signals originate, was not known.

“We sought to determine which lipid kinase was responsible for generating PIP₂ in pain-sensing neurons, with the idea that inhibiting

this kinase might reduce the levels of PIP₂ and reduce signaling through pain-producing receptors,” Zylka said.

A top candidate was phosphatidylinositol-4-phosphate 5-kinase type 1 γ (PIP5K1C), which had previously been shown to be expressed in the brain of mice and whose knockout decreased PIP₂ levels in the brain by ~50% compared with wild-type expression.^{3,4} Thus, Zylka’s team set out to test whether PIP5K1C contributes to PIP₂ production in the DRG.

In adult wild-type mice, Pip5k1c was expressed in nearly all DRG neurons. In *Pip5k1c* mice lacking one copy of the gene, PIP₂ levels in DRG neurons were ~50% lower than those in wild-type mice, whereas PIP₂ levels in the spinal cord and brain were comparable between the two groups, suggesting that Pip5k1c is responsible for PIP₂ production specifically in the DRG.

Next, Zylka’s team tested whether modulating the expression of *Pip5k1c* could alleviate pain. In *Pip5k1c*^{+/-} mice, signaling downstream of lysophosphatidic acid (LPA), which activates neuropathic pain; 17-phenyl trinor prostaglandin E₂ (17-PT PGE₂), which activates inflammatory pain; and capsaicin, which activates thermal pain, was lower than that seen in wild-type mice. In these mice, pain-associated behaviors induced by LPA, 17-PT PGE₂ or capsaicin injection were also decreased.

In multiple mouse models of chronic pain with insults initiated at distinct anatomical locations, *Pip5k1c* depletion reduced pain.

Finally, the team sought to test whether pharmacological inactivation of PIP5K1C could treat pain. To do this, Zylka collaborated with Jin and Stephen Frye to generate a small molecule inhibitor of the kinase. Frye is director of the Center for Integrative Chemical Biology and Drug Discovery and a professor of chemical biology and medicinal chemistry at UNC’s Eshelman School of Pharmacy.

An *in vitro* kinase activity screen of 5,000 compounds identified a lead, UNC3230, with a K_d of about 0.2 μ M against PIP5K1C. The compound was largely selective for PIP5K1C in a panel of 148 kinases.

In cultured murine DRG neurons, UNC3230 decreased LPA-induced calcium signaling compared with vehicle. In mouse models of inflammatory and neuropathic pain, intrathecal or intraplantar injection of UNC3230 dose-dependently inhibited pain-associated behavior, whereas an inactive analog did not.

The study was published in *Neuron*.

“Using a trifecta of pain models, the authors show substantial analgesic activity,” said pain expert William Schmidt. “Turning off pain signals before they reach the CNS will hopefully have less or different side effects from available drugs.” Schmidt is president of **NorthStar Consulting LLC**, VP of clinical and regulatory for **Centrexion Corp.**, VP of clinical and regulatory for **Api Genesis LLC** and VP of clinical development at **EicOsis LLC**. Centrexion, EicOsis and Api Genesis are developing non-opioid therapeutics to treat pain.

“Novel, druggable targets for neuropathic pain are rare opportunities. We hope our work motivates drug discovery efforts,” said Frye.

Taking PIP₂ to the clinic

Although the study provides proof of concept for a new druggable player in pain signaling, additional safety studies are needed given the chronic

use of pain drugs and the central role that PIP5K1C plays in synaptic function.³

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They’ve done a reasonable job so far but with limited models,” noted Schmidt. “They haven’t evaluated their hypothesis in higher species, and they haven’t had a chance to evaluate other types of functions that might be critical to the normal animal.”

He added, “They’ve done single-dose studies, and validation requires repeat-dose studies. They will also need to move from experimental pain models to pathological pain models and to use additional animal models. They have studied the effectiveness of their compound for up to seven days in each pain state, but they need to extend the studies and address administration, progressing to oral or parenteral delivery.”

Zylka said that a key next step is to develop PIP5K1C inhibitors with better solubility and oral bioavailability. “This will entail medicinal chemistry optimization of our existing inhibitor and several others that we identified in our initial screen,” he noted.

Frye agreed that solubility is the key hurdle they will need to overcome and added, “We will be working in other templates, so reproducing the observed effects with a different chemotype would further support PIP5K1C as a target in pain.”

In addition to expanded validation studies, Schmidt suggested that human populations with mutations in *PIP5K1C* could be tapped as a resource for exploring the viability of the target. “It would be interesting to evaluate a human cohort with heterozygosity [in *PIP5K1C*], looking at pain, cardiovascular and gastrointestinal function. This could help them rule out side effects that have been seen with other types of drugs,” he said.

Schmidt told *SciBX* that he was excited about the discovery of a putative new target in pain but emphasized that this is only the beginning of validation for PIP5K1C as a clinically relevant target.

“The convergence point idea is a strength as long as they can demonstrate that being at this position in these pathways will not interrupt other critical functions.

The UNC scientists detected no differences between adult *Pip5k1c*^{+/-} mice and wild-type mice in assays that assessed general motor function. The scientists also detected no defects in synaptic transmission or synaptic vesicle trafficking in the *Pip5k1c*^{+/-} mice.

One potential cause for concern is the association between homozygous mutations in *PIP5K1C* and lethal contractural syndrome type 3.⁵ The syndrome is lethal at or around birth and is characterized by joint contractures and muscle atrophy.

“Homozygous mutations are lethal in animals and humans,” said Schmidt. “Could you develop this type of musculoskeletal impairment with a small molecule antagonist in an adult? This could limit the dose or the timing of administration to temporary applications.”

Schmidt emphasized that the consequences of inhibiting PIP5K1C remain to be evaluated in more detail. “If it doesn’t have adverse effects, wonderful. If it does, they might consider ways to limit access of a drug to critical organs, perhaps by targeted administration.”

Patent applications covering the PIP5K1C inhibitors and the application of PIP5K1C inhibitors for treating pain have been filed and are available for licensing from UNC’s Office of Technology Development.

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