Targeting Sodium Channels for Pain Relief

The race to develop analgesic drugs that inhibit sodium channel $Na_V1.7$ is revealing a complex sensory role for the protein.

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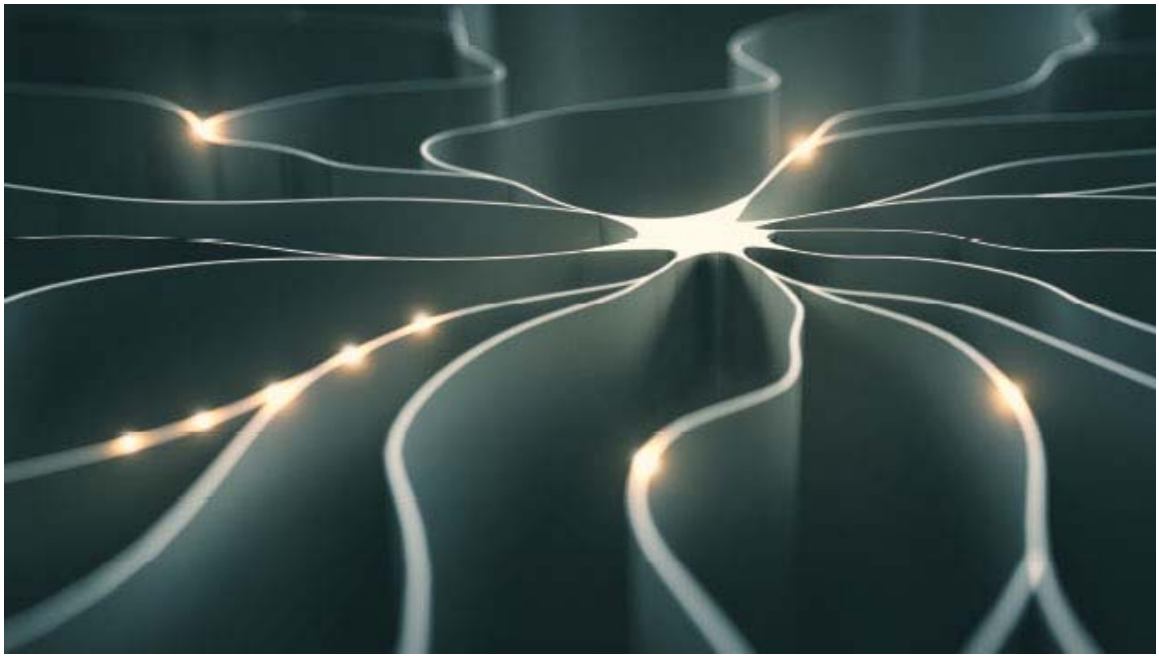


Neurobiologist John Wood has long been interested in how animals feel pain. His research at University College London (UCL) typically involved knocking out various ion channels important in sensory neuronal function from mouse models and observing the effects. But in the mid-2000s, a peculiar story about a boy in Pakistan opened up a new, and particularly human-centric, research path.

The story was relayed by Geoff Woods, a University of Cambridge geneticist. “Geoff had been wandering round Pakistan looking for consanguineous families that had genes contributing to microcephaly,” Wood recalls. During his time there, “somebody came to see him and said that there was a child in the marketplace who was damaging himself for the tourists—and was apparently pain-free.” The boy would regularly stick knives through his arms and walk across burning coals, the stories went.

Wood’s group at UCL had just published a paper describing a similarly pain-insensitive phenotype in mice genetically engineered to lack the voltage-gated sodium channel $Na_V1.7$ in pain-sensing neurons, or nociceptors. $Na_V1.7$ controls the passage of sodium ions into the cell—a key step in membrane depolarization and, therefore, a neuron’s capacity to propagate an action potential. Wood’s postdoc, Mohammed Nassar, had shown that mice lacking functional $Na_V1.7$ in their nociceptors exhibited higher-than-

normal pain thresholds; they were slower to withdraw a paw from painful stimuli and spent less time licking or biting it after being hurt.[1] Having read the study, Cambridge's Woods reached out to the group in London to discuss whether this same channel could help explain the bizarre behavior of the boy he'd heard about in Pakistan.

The two labs decided to collaborate to learn more about the human phenotype, now known as congenital insensitivity to pain (CIP). Although the boy from the marketplace had died before researchers could study him—he'd sustained fatal head injuries jumping down from the roof of a building on his 14th birthday—Woods located three other Pakistani families with members who displayed the pain-free phenotype. Using a genome-wide scan, a team led by Cambridge postdoc James Cox identified mutations in all three families within a region of *SCN9A*, the gene that codes for $Na_V1.7$. The findings, published in 2006,[2] suggested that "$Na_V1.7$ is absolutely required for humans to feel most sorts of pain," Wood says. "That was a bit of a breakthrough."

The gene itself was not unfamiliar to pain researchers, however; previously, it had been implicated in a different human pain syndrome. In 2004, Chinese researchers linked specific gain-of-function mutations in *SCN9A* to inherited erythromelalgia (IEM)—a condition with symptoms at the opposite end of the spectrum from those of CIP.[3] Patients with IEM, also known as "man on fire" syndrome, "feel searing, excruciating, scalding pain in response to mild warmth," says Stephen Waxman, a neurologist at Yale School of Medicine and the Veterans Affairs Hospital in Connecticut. Triggers include "putting on a sweater, wearing shoes, going into a room at 68 degrees Fahrenheit." Later that year, Waxman's group showed why: $Na_V1.7$ in people with IEM is unusually active, and makes pain-signaling neurons respond to even mild stimuli.[4] "Initially, we found the gain-of-function mutations, which cause excruciating pain, and two years later, the loss-of-function mutations were found" by the UK team, says Waxman. "It's unusual to be dealt a hand that complete."

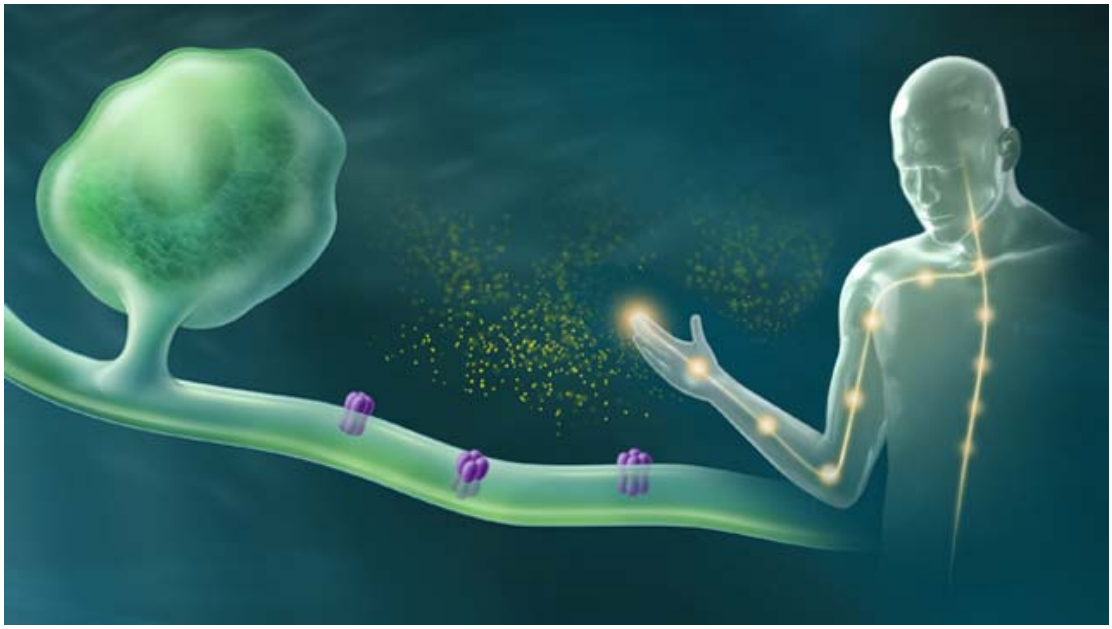
In addition to providing explanations for two specific pain syndromes in humans, the results attracted the attention of researchers working on treatments for a much broader range of conditions associated with neuropathic pain. A chronic pain state associated with nerve fibers rendered dysfunctional by injury or disease, neuropathic pain is virtually untreatable—even powerful analgesics such as opioids have mixed success in pain management, not to mention a tendency to induce dependence. "The existing drugs either don't work, work only partially, or have unacceptable side effects," says Waxman. "There's a desperate need for better medications."

With the work on CIP and IEM providing a clearer picture of the sodium channel's function, researchers hoped to create improved pain medications by designing $Na_V1.7$ blockers to produce complete analgesia in patients. Scientists also figured that $Na_V1.7$'s almost exclusive presence in peripheral neurons—a property shared by only two other voltage-gated sodium channels in humans, $Na_V1.8$ and $Na_V1.9$—would allow compounds targeting the protein to steer clear of the central nervous system, and thus avoid dependence and other side effects common to opioids.

But the last 10 years have not been smooth sailing for $Na_V1.7$ drug development. A wave of early attempts from the pharmaceutical industry to inhibit the channel were unsuccessful, in part because it has been difficult to design molecules that can block just $Na_V1.7$ and not closely related ion channels that play critical roles outside pain sensing. Moreover, there's a growing appreciation that there's more to the protein than meets the eye. "In principle, it may be a good target," says geneticist Ingo Kurth, who directs RWTH Aachen University's Institute for Human Genetics in Germany. "However, from what we have seen in recent years, [exploiting] it seems to be really complex and difficult."

Closing the gate

Like the other eight proteins in the voltage-gated sodium channel family, $Na_V1.7$ is made up of four voltage-sensing transmembrane domains surrounding a central pore through which sodium ions pass into the neuron. Blocking that pore with a small-molecule drug has been a reliable route to analgesia for well over a century. "We've had sodium channel blockers for donkey's years," says Irina Vetter, deputy director of the Centre for Pain Research at the University of Queensland in Australia. The "prototypical sodium channel blocker," she adds, is cocaine, isolated in 1855 from the leaves of the coca plant—for centuries chewed for their stimulant properties by native South Americans. The compound is still used as a local anesthetic for purposes such as orofacial surgery.



A PAINFUL PATHWAY: Since the mid-2000s, the voltage-gated sodium channel $Na_V1.7$ has emerged as a promising target for a new class of analgesics. $Na_V1.7$ controls the passage of sodium ions into sensory neurons. Hyperactivity in $Na_V1.7$ is associated with increased firing in pain-sensing neurons—and thus agony even in the absence of painful stimuli—while deletion of the channel appears to cause pain insensitivity.

But there's a problem with this sort of drug when it comes to broader applications. The ion-conducting pore targeted by many sodium channel blockers, including several currently in clinical studies, "is extremely well-conserved" across the Na_V protein family, Vetter explains. "That particular part of the channel is almost identical between all the different subtypes, so it's very difficult to find drugs that selectively block one or the other."

This lack of specificity is an obstacle for researchers trying to design therapeutics to systemically treat neuropathic pain, because other sodium channel family members are important for diverse physiological functions. For example, "if you inhibit $Na_V1.5$ in cardiac tissue, you'll end up with a sort of arrhythmia, or worse," says Les Miranda, executive director of research in therapeutic discovery for Amgen. "If you inhibit [$Na_V1.4$] in muscle tissue, you'll end up with partial paralysis. So clearly, if you're interested in 1.7, you've got to make sure you've got a molecule that does not touch 1.5 or 1.4 or some of the other '1.X' family members."

With this selectivity requirement in mind, many groups have started investigating molecules that target not the channel's pore, but the outer, voltage-sensing domains, which tend to be less conserved between Na_V subtypes. Some small molecules such as aryl sulfonamides, for example, inhibit the domain IV voltage sensor on $Na_V1.7$, and thus prevent the channel from opening in response to changes in voltage. Researchers from Xenon Pharmaceuticals and Genentech recently showed that some members of this class of compounds had good specificity for $Na_V1.7$ over cardiac $Na_V1.5$ and produced analgesia in mouse models of acute and inflammatory pain—although they show poorer specificity for their target over two channels present predominantly in the brain, $Na_V1.2$ and $Na_V1.6$.[5]

Waxman's group, in collaboration with Pfizer, showed in 2016 that a synthetic aryl sulfonamide dubbed PF-05089771 could reduce neuronal hyperactivity in a "pain-in-a-dish" model—sensory neurons grown from induced pluripotent stem cells derived from patients with IEM mutations. The drug was also well-tolerated as a single oral dose in a randomized, double-blind trial of five IEM patients, and temporarily reduced the magnitude and duration of pain attacks in most participants—although the authors noted that there was a high degree of variability in responses among patients.[6]

There's also growing interest in non-small molecules as potential $Na_V1.7$ blockers. In 2014, a group at Duke University Medical Center published a claim that monoclonal antibodies could be designed to selectively target $Na_V1.7$, and provide analgesia in mice.[7] However, the results have not yet been replicated, and for the most part, the approach has not bred much success, notes Miranda. "We have struggled to find antibodies that bind, let alone inhibit, ion channels," he says.

More-promising results have come from experiments with peptides—in particular, ion-channel modulators identified by screening toxins from venomous arthropods. As relatively large molecules, many toxin peptides naturally target "not the pore of the channel, to block ion flow, but the mechanism by which the channel is actually

activated," explains University of Queensland biochemist Glenn King, who has worked on venom-derived ion-channel blockers with Vetter, Waxman, and Wood. Like aryl sulfonamides, certain tarantula toxins selectively bind to one of $Na_V1.7$'s four voltage-sensing domains, and can lock the channel in a closed or inactivated state by making it voltage-insensitive.

Several industry groups, including Miranda's team at Amgen, are developing engineered versions of peptides that can capture the selectivity of these toxins and produce pain insensitivity in animal models. One of Janssen Pharmaceuticals's latest drug candidates—a tarantula-inspired synthetic peptide—binds to and inhibits voltage-sensing regions of $Na_V1.7$, keeping the channel from opening regardless of changes in voltage. A study published last year demonstrated that the peptide almost completely suppressed pain behaviors in rats.[8] Microproteins designed by Pfizer and based on another tarantula peptide, meanwhile, show an 80-fold and 20-fold selectivity for $Na_V1.7$ over $Na_V1.2$ and $Na_V1.6$, respectively.[9]

As a result of these efforts, the problem of drug specificity is well on the way to being solved, says William Catterall, a pharmacologist at the University of Washington in Seattle. "A number of companies have succeeded in making compounds that are surprisingly specific for $Na_V1.7$," he says, noting that such molecules are also invaluable tools in the study of sodium channel function itself. "It's a very difficult task, and it's a great credit to the pharma companies to be able to do that."

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Back to basics

Given the broad array of molecules in development and the trend toward ever more-selective $Na_V1.7$ inhibitors, the lack of clinical success from the field over the past decade has surprised and dismayed many researchers. "People have been working on this

since 2006," says King. "We still don't have anything in the clinic, and we still don't have clear answers as to why these molecules sometimes work and sometimes don't work. . . . It's proven to be a tremendously more difficult task than everyone appreciated."

Certainly, some of the delay comes from the sheer awkwardness of working with large, membrane-bound proteins such as $Na_V1.7$. The first high-resolution description of a voltage-gated sodium channel's structure was a breakthrough made only in 2011 by Catterall and his colleagues—and that was in a bacterium.[10] "Just getting your hands on the protein is challenging," says Amgen's Miranda. "It's very difficult for us to isolate; it's difficult for us to get cells to express it."And while Waxman's group has made headway using human cells for drug screening, most groups rely heavily on mouse and rat models, which often pose problems at the drug validation stage due to behavioral and physiological differences in pain sensing between rodents and people. From a structural perspective, human $Na_V1.7$ may be more different from rodent $Na_V1.7$ than it is from other human sodium channels, Catterall says. As a result, "when you home in very specifically on differences between sodium channels [in one animal], you end up with molecules that are so specific they're not very good at inhibiting the channel [in a different animal]."

But there's more at play than just the issue of translating animal research into humans: even molecules considered to be highly selective for human $Na_V1.7$ have produced effects that don't come close to the phenotype of the boy at the Pakistani marketplace. "The human genetic data says that if you inhibit that channel, you should be able to block all types of pain," says King. "But we now have very good inhibitors of the channel, and that's certainly not true. It's more complex than the human genetic studies would have suggested."

Research in the last few years has revealed glimpses of that complexity. For starters, there's a growing appreciation that the channel may play a role in sensory pathways that ostensibly have nothing to do with pain. An early mystery in Wood's lab at UCL, for example, was that mice lacking $Na_V1.7$ from all the cells in their bodies—not just the pain-sensing neurons—died shortly after birth. $Na_V1.7$ knockout humans, by contrast, have no obvious phenotypic abnormalities except, of course, CIP. It wasn't until a few years ago that researchers discovered that $Na_V1.7$ is also present in olfactory neurons, and its knockout causes anosmia—a mild defect in humans but a life-threatening one for lab mice, which rely on smell to find food and potential mates.[1]

The distribution of the protein throughout the body is also a subject of uncertainty. Although $Na_V1.7$'s predominant presence in peripheral neurons was initially highlighted as a therapeutic advantage, "if you look at the sensory neurons that convey information about tissue damage, their terminals are actually inside the blood-brain barrier, in the central nervous system," says Wood. "We think there's quite a lot of action of $Na_V1.7$ at these central terminals, and it may be that drugs have to get there to be useful." Companies such as Amgen are trying to figure out what effect central nervous system delivery might have on $Na_V1.7$ blockers' analgesic potential, Miranda notes.

More complexity comes from the recent suggestion that $Na_V1.7$'s effects on sensory neurons may go far beyond controlling the passage of sodium ions. While working with knockout mice a few years ago, Wood says, his group made a surprising discovery that implies a role for the channel in regulating transcription. "We found that in the $Na_V1.7$ knockout, opioid peptides—the enkephalins—are upregulated," he says. The group hypothesized that the CIP phenotype in patients lacking functional $Na_V1.7$ from birth might therefore come not only from the lack of sodium channel activity, but also from a boost in endogenous opioid signaling—something that analgesic drugs would have to reproduce to be successful.[12]

To test this theory, Wood and his colleagues administered an opioid blocker to a woman with CIP. "We treated her with naloxone, and she could begin to detect unpleasant stimuli" for the first time, says Wood. "She was thrilled."

More recently, in collaboration with Vetter, King, and Waxman, Wood found that administering a highly selective $Na_V1.7$-blocking spider toxin called Pn3a—which is not by itself analgesic—alongside subtherapeutic doses of opioids produced profound analgesia in mouse models of inflammatory pain, suggesting that a combinatorial drug approach might finally recapture the pain insensitivity researchers are pursuing.[13] "We're very, very keen to carry out proof-of-concept studies in healthy humans," Wood says.

The opioid signaling hypothesis is far from being widely accepted in the field at this point, although several researchers who spoke to *The Scientist* suggested it may well turn out to be correct. Nevertheless, the concept reflects a growing appreciation of the nuance in the $Na_V1.7$ story. Even Cambridge's Geoff Woods, who identified those early extreme phenotypes in $Na_V1.7$-null humans, recently published a case report describing a woman born with CIP who suddenly began reporting pain symptoms after giving birth to her child. "Her case strongly suggests that at least some of the symptoms of neuropathic pain can persist despite the absence of the $Na_V1.7$ channel," the authors write.[14]

King and other researchers, meanwhile, are investigating the possibility that blocking $Na_V1.7$ in combination with at least one other voltage-gated sodium channel is a more effective route to analgesia than targeting $Na_V1.7$ alone. "I think until we really fully understand what's going on, it's going to be really hard to develop molecules that work as well as we might have hoped," King says.

Still, with the attention that the last decade of research has brought to pain-linked sodium channels such as $Na_V1.7$, many researchers are cautiously optimistic that understanding the protein's biology and developing effective molecules against it are achievable goals. "That conviction really drives innovation in this area," Miranda says. "I think given our learning curve around the engineering of molecules, and what we've learned about how to handle and characterize $Na_V1.7$, we are going to be making—collectively, across the industry—progress against $Na_V1.7$ in the near future."

ALTERNATIVE TARGETS?

$Na_V1.7$ isn't the only voltage-gated sodium channel being investigated for novel pain treatments. Channels $Na_V1.8$ and $Na_V1.9$ are also predominantly expressed in the peripheral nervous system and have been associated with pain syndromes of their own.

In 2012, an international team of researchers identified two gain-of-function mutations in *SCN10A*—the gene coding for $Na_V1.8$—that altered the channels' activity in a way that rendered sensory neurons hyperexcitable, leading to painful neuropathy (*PNAS*, 109:19444-49). And a couple of years ago, researchers at Pfizer described a $Na_V1.8$-blocking compound that reduced neuronal excitability in human neurons in vitro and apparently produced analgesia in rodent models of inflammatory and neuropathic pain (*Br J Pharmacol*, 172:2654-70, 2015). Scientists have not yet found any loss-of-function mutations leading to a phenotype analogous to pain insensitivity in people with certain mutations in *SCN9A*, the gene coding for $Na_V1.7$.

$Na_V1.9$, by contrast, has been associated with both hypersensitivity and insensitivity to painful stimuli. Like its relatives, $Na_V1.9$ can be rendered hyperactive by gain-of-function mutations in its gene, *SCN11A*. A few years ago, Ingo Kurth at RWTH Aachen University in Germany described a novel mutation in this gene that causes pain insensitivity. Counterintuitively, this particular point mutation causes hyperactivity in $Na_V1.9$ channels; instead of leading to increased pain signaling, the aberrant channel activity means neuronal membranes are consistently depolarized. As a result, cells are unable to generate normal action potentials or communicate properly with other neurons (*Nat Genet*, 45:1399-404, 2013).

Unfortunately, this state of pain-suppressing hyperactivity is likely to be even harder to recreate than $Na_V1.7$-linked insensitivity to pain. For $Na_V1.9$, "the [molecular] mechanisms are very similar between the pain insensitivity phenotype and [the phenotype associated with] more pain," Kurth explains. "It'd be quite difficult to find a drug and concentration to produce a phenotype for pain loss." For now, $Na_V1.7$ remains the leading target among voltage-gated sodium channels for the development of novel analgesics.

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