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Tiny proteins have outsized influence on nerve health

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Mutations in small proteins that help convey electrical signals throughout the body may have a surprisingly large effect on health, according to results of a new Johns Hopkins study study published in *Proceedings of the National Academy of Sciences* in December using spider, scorpion and sea anemone venom.

The tiny conduits carrying those electrical signals are sodium channels that are vital to our well-being—they trigger action potentials, or spurts of electrical energy that course from body to brain to deliver messages that invoke feelings like pain or temperature sensitivity. When such channels go awry, they contribute to a slew of diseases, one of which is epilepsy.

In the new research, Frank Bosmans, Ph.D., an assistant professor of physiology at the Johns Hopkins University School of Medicine, has found that auxiliary "helper" proteins that interact with sodium channels also play a crucial role. And that, he says, could affect drug development for epilepsy, neurological diseases, muscular disorders and pain syndromes.

"Nobody had thought these tiny molecules that don't even form the main sodium channel were capable of changing the response of the channel to certain compounds," Bosmans says. "But in what we consider a new concept, these auxiliary subunits can be considered as drug targets."

Over the past few decades, there have been hints that these auxiliary

proteins were influencing sodium channels, but few analyzed the problem very closely. John Gilchrist, a graduate student in Bosmans' lab, began evaluating each of the four proteins, one at a time.

Gilchrist engineered frog eggs that made sodium channels and exposed them to the toxins released by tarantulas, scorpions, wasps and sea anemones, an extension of Bosmans' earlier doctoral research studying the effect of animal venoms on sodium channels. He found that one auxiliary protein in particular, beta4, altered the whole sodium channel system. When exposed to tarantula venom, for instance, tissue in the presence of beta4 showed decreased sensitivity in the sodium channels, meaning that the protein changed the way the nerve fired. This denotes that if a human got bit by a tarantula in a region where beta4 was active, the whole experience might be just a little less painful, says Bosmans.

To figure out what was going on in the altered channels, Bosmans needed to know what the protein looked like, he says. He contacted Filip Van Petegem, a crystallographer at the University of British Columbia in Vancouver, Canada. Van Petegem was able to map the 3-D structure of beta4 down to 1.7 angstroms, the highest possible resolution. Crystal structure in hand, Bosmans could now mutate beta4 and watch what happened.

Purely by chance, Van Petegem had already started that mutation process. To diagram the crystal, Van Petegem had been forced to substitute one protein for another due to quirks in the test system. Bosmans found that the tiny mutation thwarted beta4's interaction with the sodium channel system.

That finding promptly overturned conventional wisdom into how these proteins behave, Bosmans says.

Back in 1998, Bosmans says, physicians determined that a mutation in

the beta1 protein seemed to be triggering a case of epilepsy. Epilepsy has hundreds of causes. It was known at the time that a chemical bridge within the sodium channel held the beta proteins together. If that bridge, known as a disulfide bond, is broken, the proteins fall apart. The physicians theorized that the mutation they found must have destroyed the bridge along with their accompanying proteins. That broken bridge theory has remained dominant ever since.

But when Bosmans introduced that same mutation in beta4, the structure stayed intact. The changes he saw were much more subtle. The position of the protein Van Petegem had mutated changed slightly so that it was farther away from the channel. And only when that mutated crystal was exposed to a toxin did beta4 lose its ability to communicate with the sodium channel.

Bosmans says that even with evidence of the auxiliary proteins' importance mounting, such as in the epilepsy study, drug developers have continued to ignore the proteins rather than treatment opportunities. Most efforts to develop new drugs to treat epilepsy still focus exclusively on modifying the sodium channels, which don't need the beta proteins to operate. But Bosmans believes this is only part of the story.

His new finding suggests that such an approach is shortsighted, because mutations in these beta proteins may very well be causing the disease at hand. Drugs that target the beta proteins have the potential to deliver a much more focused treatment, he says.

"That's one of the new concepts that we're trying to launch—keep an eye on these little guy proteins, because they are important. If they have a mutation in them, they can cause a disease," Bosmans says.

More information: Crystallographic insights into sodium-channel

modulation by the β4 subunit. John Gilchrist, Samir Das, Filip Van Petegem, Frank Bosmans (2013). *Proceedings of the National Academy of Sciences* . Epub ahead of print. DOI: 10.1073/pnas.1314557110