Why poison dart frogs don't poison themselves

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P. terribilis. Credit: Wikimedia Commons/Micha L. Rieser.

(Phys.org)—A pair of researchers with the State University of New York has found the source of poison dart frogs' immunity from their

own poison. In their paper published in *Proceedings of the National Academy of Sciences*, Sho-Ya Wang and Ging Kuo Wang describe testing frog muscle-derived amino acids in rat muscles to determine if one of them might be responsible for preventing muscle from seizing.

Poison dart frogs, native to Columbia, are known throughout the world for the application of their poison to blow darts as a weapon. The toxin is produced in the skin gland and one frog holds enough to kill 10 human beings at any one time—the toxin kills by reversing the openings of sodium channels in nerves, which prevents muscles from relaxing. The heart clenches to push blood through the body, but then cannot unclench, preventing it from working.

Prior research has shown that the active ingredient in the toxin is batrachotoxin. To figure out why the dart frogs do not give themselves heart attacks when they produce the chemical, the researchers introduced five naturally occurring amino acid replacements found in the frog's muscles into rat muscles. Doing so, the researchers report, made the rat muscle immune to the effects of batrachotoxin. The researchers then tested the amino acids individually until they found the one that was responsible for the change—N1584T. This finding overturns prior research results that suggested multiple factors were responsible for frog immunity—it shows that the immunity in the frogs comes from a single genetic mutation.

Unfortunately, as with the puffer fish, which was also found to have just one amino acid that protected it from harming itself, this discovery is not likely to offer a path to an antidote for those who fall prey to the effects of dart frog toxin. It might offer new data, however, for those conducting research involving using the toxin as a pain killer.

More information: "Single rat muscle Na+ channel mutation confers batrachotoxin autoresistance found in poison-dart frog Phyllobates

terribilis," by Sho-Ya Wang and Ging Kuo Wang. *Proceedings of the National Academy of Sciences* (2017).
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Abstract
Poison-dart Phyllobates terribilis frogs sequester lethal amounts of steroidal alkaloid batrachotoxin (BTX) in their skin as a defense mechanism against predators. BTX targets voltage-gated Na+ channels and enables them to open persistently. How BTX autoresistance arises in such frogs remains a mystery. The BTX receptor has been delineated along the Na+ channel inner cavity, which is formed jointly by four S6 transmembrane segments from domains D1 to D4. Within the P. terribilis muscle Na+ channel, five amino acid (AA) substitutions have been identified at D1/S6 and D4/S6. We therefore investigated the role of these naturally occurring substitutions in BTX autoresistance by introducing them into rat Nav1.4 muscle Na+ channel, both individually and in combination. Our results showed that combination mutants containing an N1584T substitution all conferred a complete BTX-resistant phenotype when expressed in mammalian HEK293t cells. The single N1584T mutant also retained its functional integrity and became exceptionally resistant to 5 μM BTX, aside from a small residual BTX effect. Single and combination mutants with the other four S6 residues (S429A, I433V, A445D, and V1583I) all remained highly BTX sensitive. These findings, along with diverse BTX phenotypes of N1584K/A/D/T mutant channels, led us to conclude that the conserved N1584 residue is indispensable for BTX actions, probably functioning as an integral part of the BTX receptor. Thus, complete BTX autoresistance found in P. terribilis muscle Na+ channels could emerge primarily from a single AA substitution (asparagine→threonine) via a single nucleotide mutation (AAC→ACC).