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RESEARCH NEWS

Grammotoxin increases its toxic behavior

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This *JGP* study (Collaço et al. https://doi.org/10.1085/jgp.202413563) reveals that in addition to voltage-gated Ca^{2+} and K^+ channels, ω-grammotoxin-SIA also inhibits voltage-gated Na^+ channel currents.

ω-Grammotoxin-SIA (GrTX-SIA) is a 36-amino acid peptide that is present in the venom of the Chilean rose tarantula and was initially shown to inhibit the opening of several voltage-gated Ca^{2+} (Ca_V) channels (1). A later study revealed that GrTX-SIA similarly inhibits voltage-gated K^+ (K_V) channels by binding to a conserved structural motif present in the voltage-sensing domains (VSDs) of both K_V and Ca_V channels (2). In this issue of *JGP*, Collaço et al. report that GrTX-SIA is even more promiscuous in its inhibitory activity, as it can also bind to two of the four VSDs found in voltage-gated Na^+ (Na_V) channels (3).

At the time when GrTX-SIA was originally discovered, small peptide toxins were commonly thought to be highly specific inhibitors whose activity was limited to a handful of closely related ion channels. In recent years, however, it has become clear that many toxins are, in fact, quite promiscuous and capable of targeting a large number of different channels.

"From an evolutionary perspective, it makes sense for organisms to produce peptides that are as toxic as possible and have a wide array of targets," says Frank Bosmans, a professor at VUB and UGent in Belgium. As a postdoc, Bosmans found that hanatoxin, a well-characterized tarantula toxin that modifies the gating of K_V channels, is an even more potent inhibitor of Na_V channels (4). Similar to GrTX-SIA, hanatoxin binds to a structural motif—spanning the S3 and S4 transmembrane helices—that is found in the VSDs of Ca_V, K_V, and Na_V channels. Bosmans and colleagues, including postdoctoral researcher Rita de Cássia Collaço, therefore wondered whether GrTX-SIA might also bind to Na_V channel VSDs and inhibit Na_V channel gating.

To address this question, Collaço et al. expressed eight Na_V channel subtypes ($Na_V1.1$–$Na_V1.8$) in *Xenopus* oocytes and tested the effect of GrTX-SIA on channel conductance (3). GrTX-SIA inhibited all eight channels to some degree, with $Na_V1.6$ being the most susceptible subtype.

While analyzing the effect of GrTX-SIA on $Na_V1.6$ gating, the researchers noticed that the toxin inhibits $Na_V1.6$ in a biphasic manner. Channel conductance declines rapidly in the first 2 min after toxin application, but this is followed by a second, slower phase of inhibition that further reduces $Na_V1.6$ currents. "That was a hint that there might be two distinct binding sites on the channel," Bosmans says.

To determine whether GrTX-SIA binds to one or more of the four VSDs present in $Na_V1.6$, Collaço et al. used a series of chimeric constructs in which the S3–S4 regions of each $Na_V1.6$ VSD are transplanted into the VSD of the $K_V2.1$ channel. Chimeras containing the S3–S4 regions of $Na_V1.6$ VSDI or VSDIII were unaffected by GrTX-SIA. In contrast, chimeras containing the S3–S4 regions of $Na_V1.6$ VSDII or VSDIV were strongly inhibited upon toxin application. Further experiments suggested that VSDII is a low-affinity binding site for GrTX-SIA that likely mediates the later, slower phase of inhibition. VSDIV, on the other hand, is a higher affinity site that probably promotes the initial, rapid onset of inhibition. Though VSDIV has long been known to mediate Na_V channel inactivation, Collaço et al.'s findings add to data suggesting that it is also crucial for channel opening. "GrTX-SIA prevents channel opening and its highest affinity target is VSDIV," Bosmans says. "So, that suggests that VSDIV is involved in channel opening."

Rita de Cássia Collaço and Frank Bosmans.

To confirm their findings, Collaço et al. used molecular modeling to identify several glutamate resides in $Na_V1.6$ VSDII/IV that were likely to be crucial for the interaction with GrTX-SIA. Mutating these residues to lysine greatly reduced $Na_V1.6$'s sensitivity to the toxin.

However, while individual residues can modulate GrTX-SIA's affinity for any particular target, the toxin's ability to recognize a conserved, 3-D structure in VSDs allows it to inhibit a wide range of voltage-gated ion channels. The ability of GrTX-SIA and other toxins to bind lipids and partition into the plasma membrane is also likely to contribute to their promiscuity.

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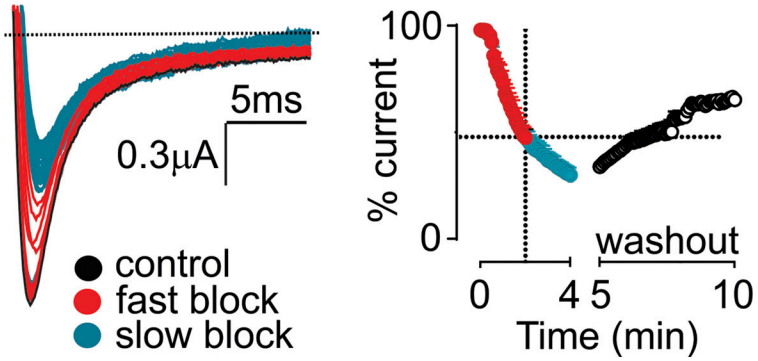

Collaço et al. found that, because it can bind to a conserved structural motif in VSDs, the tarantula toxin GrTX-SIA can inhibit Na_V channels as well as K_V and Ca_V channels. The toxin binds to two of the four VSDs in $Na_V1.6$, resulting in a biphasic pattern of inhibition characterized by an initial, fast phase (red) and a later, slower phase (blue).

Yet, though they may not be as specific as once thought, Bosmans insists that toxins are still of great interest to the research community. "They are still valuable tools to study ion channels as well as potential lead compounds for drug development," he says.

References

1. Lampe, R.A., et al. 1993. *Mol. Pharmacol.* 44: 451–460.
2. Li-Smerin, Y., and K.J. Swartz. 1998. *Proc. Natl. Acad. Sci. USA.* https://doi.org/10.1073/pnas.95.15.8585
3. Collaço, R.C., et al. 2024. *J. Gen. Physiol.* https://doi.org/10.1085/jgp.202413563
4. Bosmans, F., et al. 2008. *Nature.* https://doi.org/10.1038/nature07473