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## **Pinnatoxins A and G potently and reversibly block transmission at the skeletal neuromuscular junction in vivo and in vitro**

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The dinoflagellate *Vulcanodinium rugosum*, first isolated from Ingril, a French Mediterranean lagoon, is known to produce the pinnatoxins (PnTXs) and the portimines. PnTXs (A-H) constitute an emerging family of phycotoxins belonging to the cyclic imine group<sup>1,2</sup>. Interest has been focused on these fast-acting highly potent toxins because they are widely found in contaminated shellfish. Despite their highly complex molecular structure, PnTXs have been chemically synthesized by the Zakarian's group, and demonstrated to act on various nicotinic acetylcholine receptors (nAChRs)<sup>3,4</sup>. To the best of our knowledge, neither PnTX-A nor PnTX-G and analogs, obtained by chemical synthesis with high degree of purity (> 98%), have been studied *in vivo* or *in vitro* on adult mouse and isolated nerve-muscle preparations expressing the mature muscle-type ( $\alpha 1$ )<sub>2</sub> $\beta 1$  $\epsilon \delta$  nAChR. Our results show that PnTX-A and PnTX-G acted on the neuromuscular system of anesthetized mice and blocked the compound muscle action potential (CMAP) in a dose- and time-dependent manner with similar ID<sub>50</sub> values (dose required to block 50% of the CMAP), as determined using an *in vivo* minimally invasive electrophysiological method. The decrease of CMAP induced by both toxins *in vivo* was reversible within 6-8 h. PnTX-A and PnTX-G, applied to isolated *extensor digitorum longus* (EDL) nerve-muscle preparations, blocked reversibly isometric twitches evoked by nerve stimulation. Both toxins exerted no direct action on the contractile machinery of muscle fibers, as revealed by direct muscle stimulation. In addition, PnTX-A and PnTX-G blocked synaptic transmission at mouse neuromuscular junctions. PnTX-A aminoketone analog (containing an open form of the imine ring)<sup>4</sup> had no effect on neuromuscular transmission. These results indicate the importance of the cyclic imine for interacting with adult muscle-type nAChR.

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<sup>1</sup>Stivala CE, Benoit E, Aráoz R, Servent D, Novikov A, Molgó J, Zakarian A. 2015. Synthesis and biology of cyclic imine toxins, an emerging class of potent, globally distributed marine toxins. *Nat Prod Rep* 32: 411-435.

<sup>2</sup>Molgó J, Marchot P, Aráoz R, Benoit E, Iorga BI, Zakarian A, Taylor P, Bourne Y, Servent D. 2017. Cyclic imine toxins from dinoflagellates: A growing family of potent antagonists of the nicotinic acetylcholine receptors. *J Neurochem* 142 (S2): 41-51.

<sup>3</sup>Aráoz R, Servent D, Molgó J, Iorga BI, Fruchart-Gaillard C, Benoit E, Gu Z, Stivala C, Zakarian A. 2011. Total synthesis of pinnatoxins A and G and revision of the mode of action of pinnatoxin A. *J Am Chem Soc* 133: 10499-10511.

<sup>4</sup>Bourne Y, Sulzenbacher G, Radić Z, Aráoz R, Reynaud M, Benoit E, Zakarian A, Servent D, Molgó J, Taylor P, Marchot P. 2015. Marine macrocyclic imines, pinnatoxins A and G: Structural determinants and functional properties to distinguish neuronal  $\alpha 7$  from muscle  $\alpha 1$ <sub>2</sub> $\beta 1$  $\gamma \delta$  nAChRs. *Structure* 23: 1106-1115.

**Keywords:** compound muscle action potential, cyclic imine, emerging toxin, marine phycotoxin, mouse neuromuscular system, pinnatoxin, synaptic potential.