

# 3,4-Diaminopyridine antagonizes the block of muscle nicotinic acetylcholine receptor caused by cyclic imine toxins at isolated mouse diaphragm preparations

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Cyclic imine toxins (CiTXs) are a family of worldwide-distributed marine macrocyclic phycotoxins comprising different groups of lipophilic compounds: gymnodimines (GYMs), spirolides, pinnatoxins (PnTXs), pteriatoxins (PteTXs), portimines, prorocentrolides and spiroporocentrimine. CiTXs and the acyl derivative products of shellfish metabolism contaminate and bioaccumulate in filter-feeding bivalves, edible mollusks and other marine invertebrates and thus, represent a risk for shellfish consumers and a menace for wildlife conservation. All CiTXs studied interact and block with different affinities, embryonic ( $\alpha_1\beta\gamma\delta$ ) and mature ( $\alpha_1\beta\varepsilon\delta$ ) nicotinic acetylcholine receptors (nAChRs) expressed in skeletal muscles. 3,4-Diaminopyridine (3,4-DAP) is a well-known K<sup>+</sup>-channel blocker that prolongs the presynaptic action potential and indirectly enhances the entry of Ca<sup>2+</sup> into nerve terminals and, thereby, increases quantal ACh release at the neuromuscular junction. 3,4-DAP is used effectively to treat muscular weakness in various human myasthenic syndromes. The aim of this work was to determine whether 3,4-DAP, which increases quantal ACh release, is able to reverse the action of CiTXs on skeletal muscle expressing  $\alpha_1\beta\varepsilon\delta$  nAChRs.

The use of isolated mouse phrenic-nerve-hemi-diaphragm muscle preparations allowed monitoring nerve-evoked isometric muscle contraction in the presence of GYM-A, PnTX-A, PnTX-G and PteTX-1 and, afterwards, the action of 3,4-DAP (10-100  $\mu$ M) in the continuous presence of the respective CiTXs.

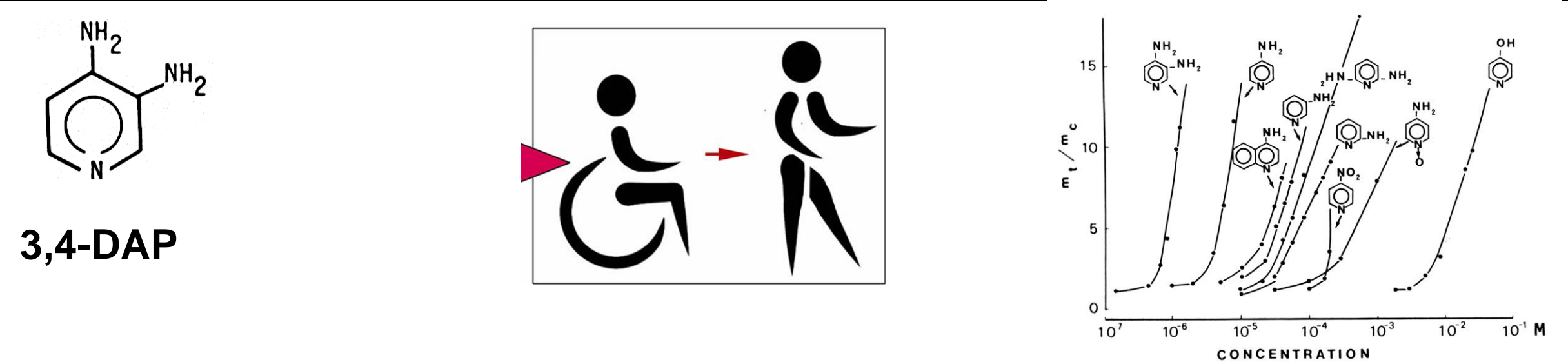
The results show that 3,4-DAP improves neuromuscular function in a time- and concentration-dependent manner and, at the highest concentration used (100  $\mu$ M), completely reverses the neuromuscular block caused by the CiTX studied. These results support the view that GYM-A, PnTX-A, PnTX-G and PteTX-1 are competitive and reversible inhibitors of muscle-type  $\alpha_1\beta\varepsilon\delta$  nAChRs. In fact, CiTXs can be typified as competing with the binding of ACh (the natural ligand) to the nAChR. The ability of 3,4-DAP to antagonize the action of CiTXs is related to the increased release of ACh that is able to displace the respective CiTX from their nAChR binding sites.

Comparison of pK<sub>a</sub> values, % of non-ionized form at physiological pH, and the Effective concentration that increases 5 times (EC<sub>5</sub>) the quantal content of the endplate potential at neuromuscular junctions

Compound	% Maximal twitch potentiation	pK <sub>a</sub> *	% non-ionized form	EC <sub>5</sub> ± SEM (μM) **
3,4-DAP	520	9.08	1.30	0.5 ± 0.02
4-AP	308	9.17	1.06	3.2 ± 0.3
4-AQ		9.17	1.06	26.0 ± 3.2
3-AP		5.78	94.32	38.2 ± 2.4
2,6-DAP		4.83	4.77	67.4 ± 5.4
2-AP	210	6.86	68.63	91.2 ± 10.2
4-NP		1.61	0.0001	410.0 ± 30.0
4-AP-N-oxycide		3.69	0.031	490.0 ± 54.5
4-HP		11.09	99.99	12.000 ± 360
2,4-DHP		6.5	16.63	Inactive
Pyridine		6.17	0.99	inactive

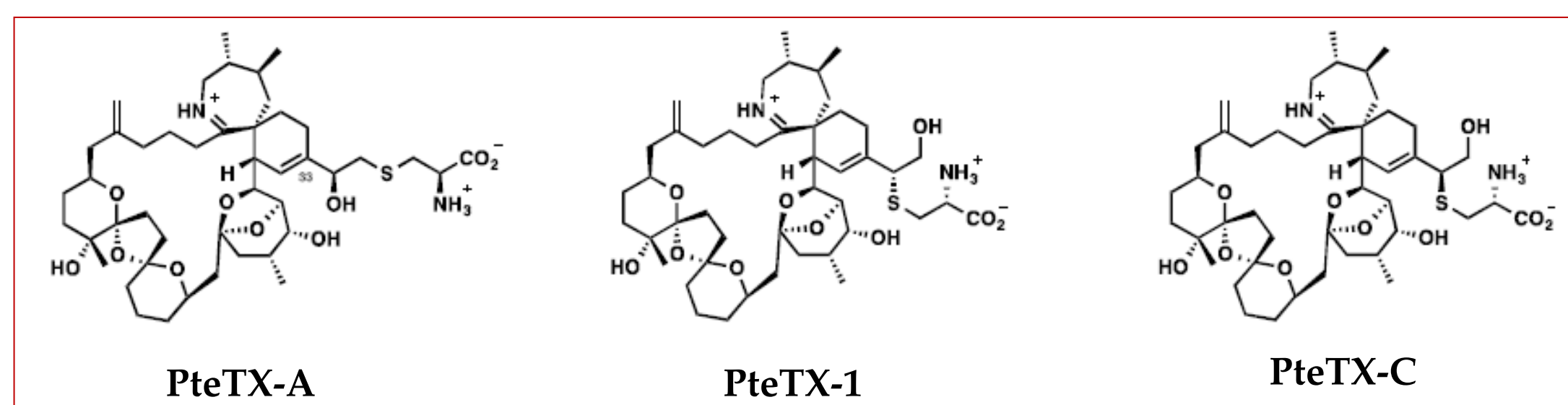
\* pK<sub>a</sub> determined values.  
\*\* EC<sub>5</sub> values were calculated from concentration-effect curves determined in an extracellular medium containing low calcium high magnesium concentration (1 mM Ca<sup>2+</sup> and 10 mM Mg<sup>2+</sup>).

3,4-Diaminopyridine (3,4-DAP) is a well-known K<sup>+</sup>-channel blocker that prolongs the presynaptic action potential and indirectly enhances Ca<sup>2+</sup> entry into nerve terminals and, thereby, increases quantal ACh release from the neuromuscular junction.

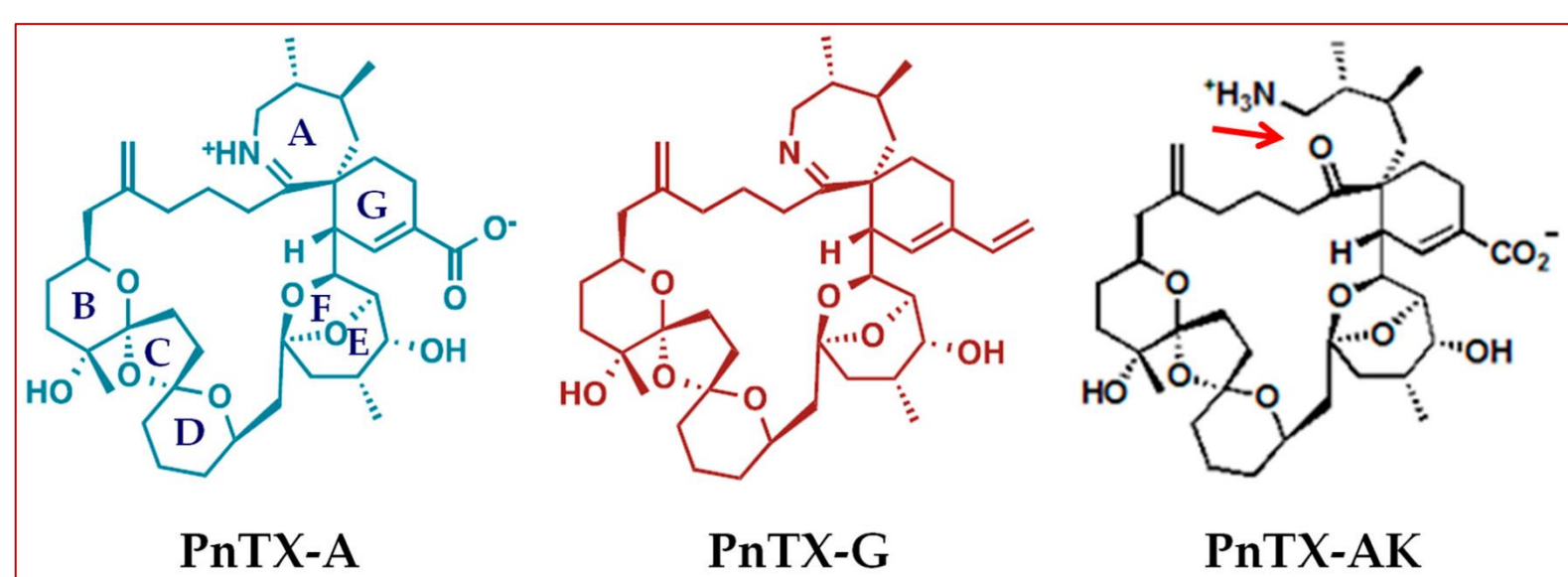
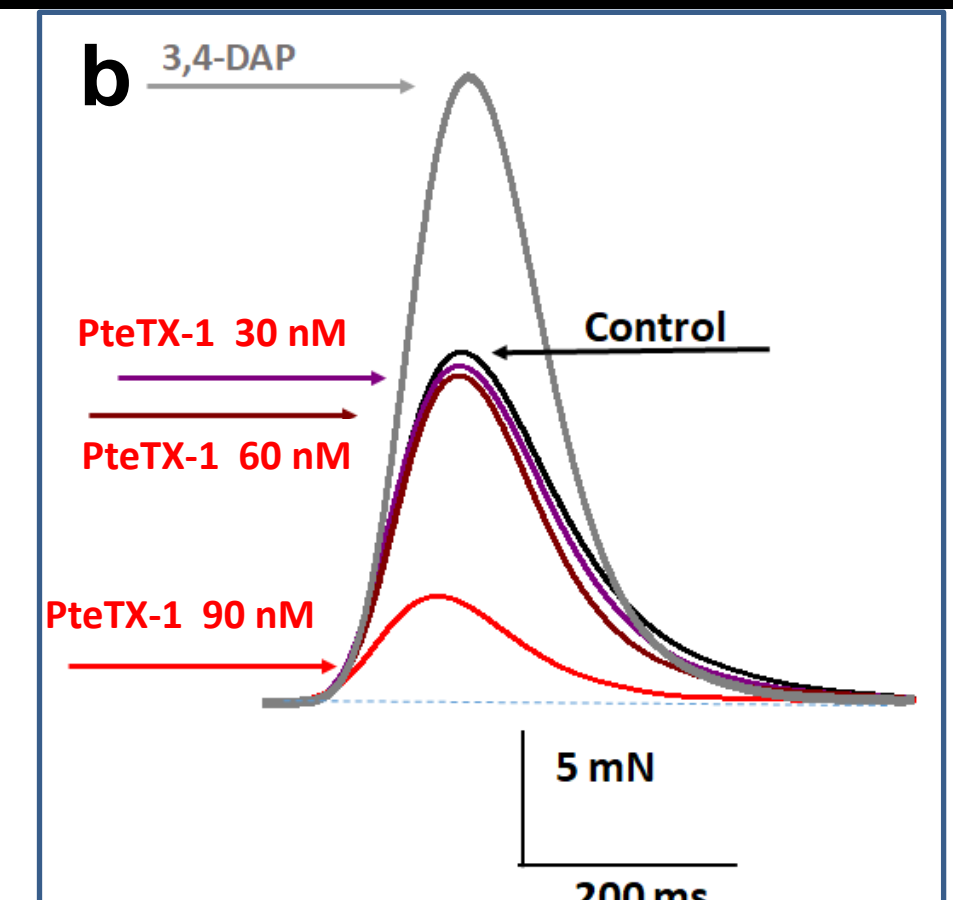
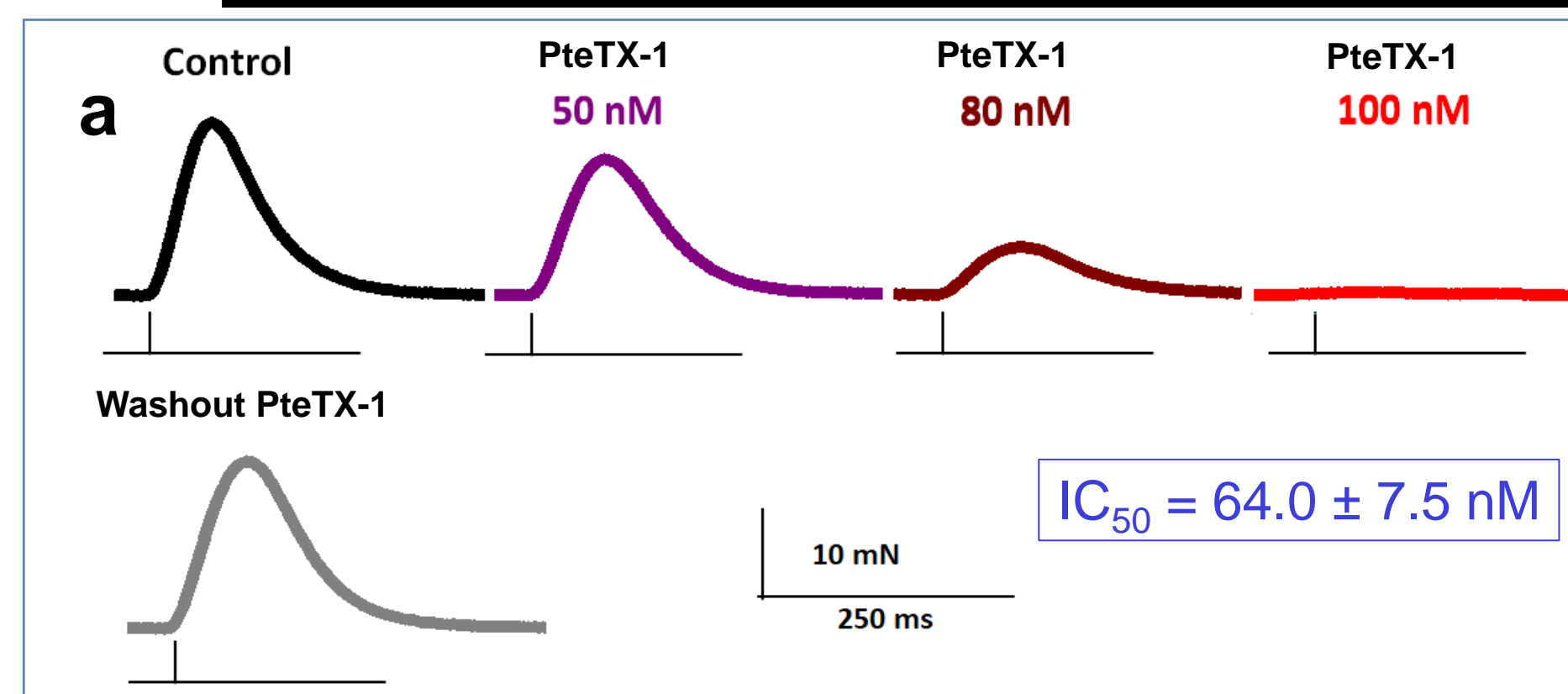


3,4-DAP has been used effectively to treat muscular weakness in various human myasthenic syndromes

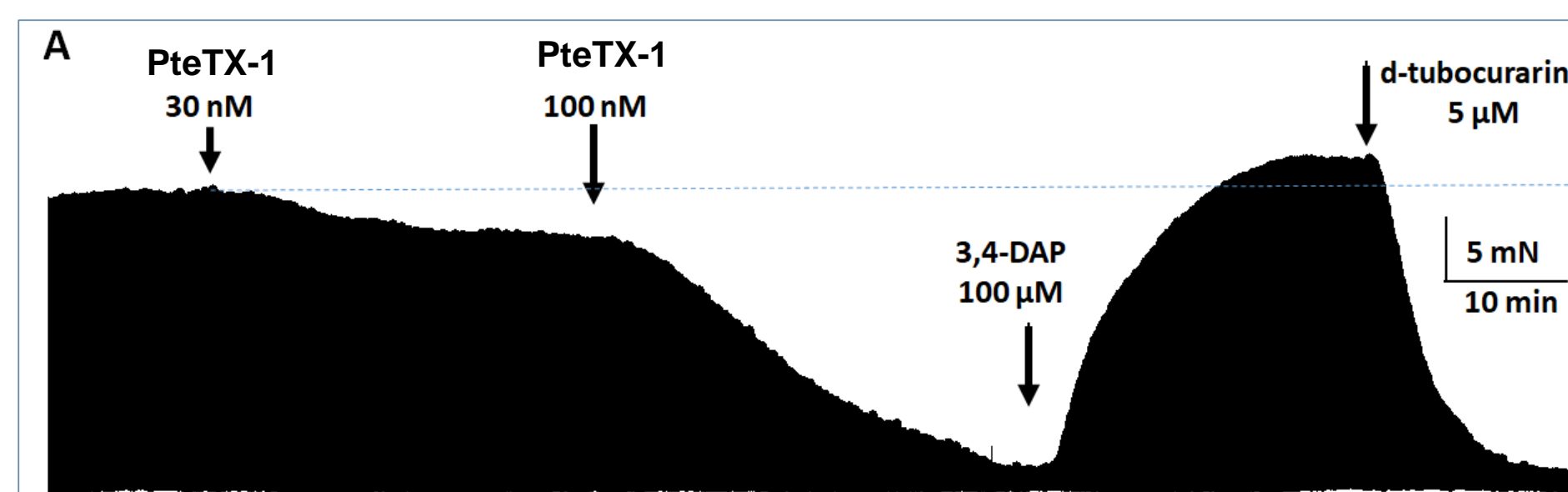
Synthetic Pteriatoxin-1 blocks nerve evoked contraction in a reversible manner (a) and the block can be reversed by 3,4-DAP (b),



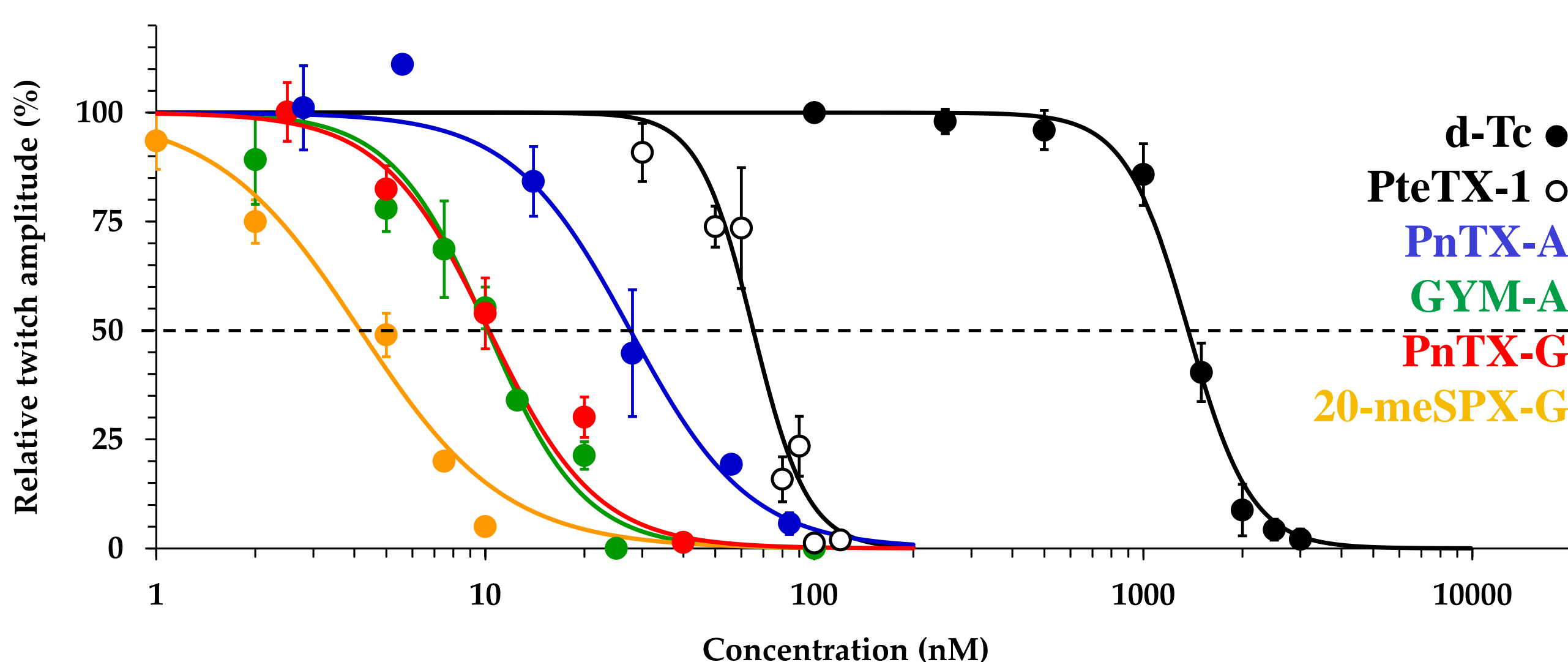
Pteriatoxins



Pinnatoxins



A. Continuous recording of nerve evoked hemidiaphragm contraction showing the affect of PteTX-1, the reversibility by 3,4-DAP and the subsequent block by d-tubocurarine



Relative twitch amplitude responses as a function of concentrations for five cyclic imine toxins compared to d-tubocurarine (d-TC). Note that cyclic imine toxins are more potent than d-TC.

## SUMMARY CONCLUSIONS

- No aminopyridine analog was found more potent than 3,4-DAP in increasing phasic quantal ACh release from motor nerve terminals of vertebrate neuromuscular junctions.
- 3,4-DAP can reverse muscle paralysis resulting from the action of the cyclic imine toxins studied (Gymnodimine A, Pinnatoxin A, Pinnatoxin G, and Pteriatoxin 1, and 20-methyl Spirolide G) supporting the view that these toxins are competitive and reversible inhibitors of muscle-type  $\alpha_1\beta\varepsilon\delta$  nAChRs
- The ability of 3,4-DAP to antagonize the action of cyclic imine toxins is related to the increased release of ACh that is able to displace the toxins from their respective nAChR binding sites,
- 3,4-DAP has the highest margin of safety compared to other pyridine analogs, and crosses less well the blood brain barrier than 4-AP. Therefore, can be recommended for reversing the block of neuromuscular transmission caused by cyclic imine toxins.