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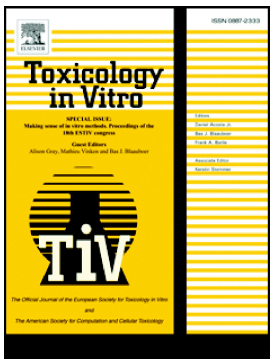

The in vitro detection of botulinum neurotoxin-cleaved endogenous VAMP is epitope-dependent

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The in vitro detection of botulinum neurotoxin-cleaved endogenous VAMP is epitope-dependent.

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Abstract

The *in vitro* potency of botulinum neurotoxin (BoNT) serotypes is often measured by monitoring cleavage of their soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) protein substrates. A frequently used method is Western blot, whereby the full-length protein and cleaved form migrate at different molecular weights. Until now, it has been extremely difficult to detect the cleaved cellular form of the SNARE protein vesicle associated membrane protein 1, 2 or 3 (VAMP1, 2 or 3) by Western blot. These VAMP isoforms are the substrates of BoNT serotypes BoNT/B, D, F and G as well as tetanus neurotoxin. Using custom made anti-VAMP antibodies against epitopes either side of the cleavage sites for BoNT/B, BoNT/D and BoNT/F, we have successfully detected the cleaved C-terminal VAMP fragment in cortical neurons. These new antibodies enable quantitative assessment of the potency of VAMP-cleaving neurotoxins by a gain of signal Western blot assay.

Keywords: botulinum neurotoxin; VAMP (vesicle associated membrane protein) cleavage; in vitro assay; Western blot.

Abbreviations: AraC (cytosine β-D-arabinofuranoside), BoNT (botulinum neurotoxin), DIV (days in vitro), ELISA (Enzyme-linked immunosorbent Assay), FBS (foetal bovine serum), HBSS (Hank's Balanced Salt Solution), PBS (phosphate buffered saline), PLO (poly-L-ornithine), RabMAb (rabbit monoclonal antibody), SDS-PAGE (sodium dodecyl sulfate

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