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Cloning, Expression, Purification and Characterization of the Twelve Subtypes of Botulinum Neurotoxin E1-E12

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Introduction: Botulinum neurotoxins (BoNTs) are classified into 10 different serotypes—BoNT/A-H, J, and X—and mosaic toxins like BoNT/CD, DC or BoNT/HA. Variants of serotypes BoNT/A, B, E, and F are categorized into subtypes A1-A8, B1-B8, E1-E12, and F1-F9.[1] The BoNT-E distinguishes from all other serotypes by showing a faster onset as well as a shorter duration of effect.[2,3] BoNT-E comprises twelve subtypes (BoNT/E1-E12), but mainly BoNT-E1 and E3 have been characterized. This project aims to produce and subsequently characterize all twelve BoNT-E subtypes.

Methods: Expression plasmids encoding BoNT/E1-E12 were generated. Subsequently, the full-length BoNT/E1-E12 were recombinantly expressed and isolated in a multiple step purification process. Biological activity of the BoNT-E subtypes was analyzed in vitro in a cell-based assay employing neurons (P19N) differentiated from mouse-derived embryonic teratocarcinoma cell line P19. Furthermore, in vitro catalytic activity was analyzed by cleavage of recombinant SNAP-25 substrate. To test epitope recognition of known nanobodies[4,5] binding of BoNT-E subtypes to these nanobodies was evaluated in pulldown assays.

Results: All BoNT-E subtypes were obtained as tag-free, fully activated di-chain proteins in high purity with the interchain disulfide bridge formed. P19N cell-based assays demonstrated biological activity of all BoNT-E subtypes in vitro. The light chain catalytic activity assay exhibits differences between the subtypes in SNAP-25 cleavage kinetics. In addition, none of the nanobodies tested recognized all BoNT-E subtypes.

Conclusions: Botulism diagnostics is highly dependent on full-coverage of all sero- and subtypes toxic to humans. The validation especially of in vitro detection methods requires all BoNT-E subtypes as test probes. Furthermore, the efficacious neutralization of the BoNT-E subtypes by botulism antitoxins and novel medical countermeasures needs to be demonstrated to ensure successful treatment of patients. In addition, the BoNT-E subtypes present a reservoir of active pharmaceutical ingredient candidates with varying pharmacological properties. Here, we present the initial work of producing and characterizing the twelve BoNT-E subtypes. In future, these subtypes will also be tested

regarding receptor recognition, neutralization by botulism antitoxins, and use in diagnostic assays.

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Keywords: Botulinum Neurotoxin E; Subtype E1-E12; P19 neurons; Nanobody

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