Calcium influx-activating action of P-EPTX-Ar1a: an isolated neurotoxin from the venom of Irian Jayan death adder

J. Chaisakul,[1] H.C. Parkington,[2] M.A. Tonta,[2] H.A. Coleman,[2] N. Konstantakopoulos[1] and W.C. Hodgson,[1]
[1]Department of Pharmacology, Monash University, Clayton, VIC 3800, Australia and [2]Department of Physiology, Monash University, Clayton, VIC 3800, Australia.

We have isolated a fraction from Irian Jayan death adder (*Acanthophis rugosus*) venom, that we have shown displays pre-synaptic neurotoxic activity in chick neuromuscular junction *via* phospholipase A_2 activity (Chaisakul *et al.*, 2010). We called this fraction P-EPTX-Ar1a. Past studies have reported that many pre-synaptic snake neurotoxins increase cellular calcium by hydrolyzing the plasma membrane and generating lysophosphatidylcholine and fatty acids (Rigoni *et al.*, 2007; Tedesco *et al.*, 2009). In this study, we investigated whether P-EPTX-Ar1a changes cytoplasmic calcium in rodent dorsal root ganglion cells (DRG).

DRGs were isolated from embryonic day (E) 19 Wistar rats or E18 Swiss mice. Cells were isolated by gentle trituration, in the absence of digestive enzymes, and the cells were plated onto 9mm poly-ornithine/laminin coated glass coverslips and rested for 2 h in DMEM/F12 containing 5% fetal calf serum, 0.5 ng/ml nerve growth factor, 1:100 dilution N2 hormone supplement and 2ng/ml glial-cell derived nerve growth factor. The cells were washed and incubated in the calcium fluorophore Fluo-4-AM at 22°C for 10 min. The cells were then continuously superfused with Hanks solution flowing at 1ml/min at 35°C, and the experiment started after a 10 min wash. The snake toxin was added to the superfusate for 4 min. In separate experiments, the patch clamp technique was used in whole cell mode to record the effects of P-EPTX-Ar1a on membrane currents in DRG cells.

P-EPTX-Ar1a, 74nM, caused a prompt increase in cytoplasmic calcium in approximately one third of DRG neurons. The smaller diameter DRG cells were more vulnerable than those of larger diameter. Bursts of cytoplasmic calcium continued to occur throughout the 4 min application of the snake toxin. Following removal of the toxin, calcium bursting abated slowly. DRG cells were then exposed to calcium-free Hanks solution for 1min prior to and during snake toxin exposure. In this situation the increase in cytoplasmic calcium in response to toxin application was delayed by about 3min. Normal (1.3mM) calcium was re-introduced during the washout period and this caused prolonged bursts in cytoplasmic calcium in 98±2% of toxin-sensitive cells. Furthermore, the increase in cytoplasmic calcium in this situation was so large (equal to or exceeding that evoked by exposure to 100 K solution for 10 s) that many cells promptly withdrew projections and discontinued association with the glass coverslip. The increase in cytoplasm calcium induced by snake toxin was unaffected by nifedipine or ω-conotoxin, which block L-type and N-type voltage-gated calcium channels, respectively. However, the response was blunted by agatoxin. Pretreatment with tetrodotoxin, which blocks voltage-gated sodium channels 1.1-1.4, 1.6, 1.7, completely prevented both the immediate increase in cytoplasmic calcium induced by snake toxin in calcium-containing Hanks solution and to the large delayed response following restoration of extracellular calcium, as described above. P-EPTX-Ar1a induced an inward current in DRG cells.

Like many other snake toxins, P-EPTX-Ar1a increases cytoplasmic calcium in neurons. The response is very difficult to reverse and the extent of the increase in cytoplasmic calcium can be sufficient to cause cell death within minutes. The underlying mechanisms may involve alteration of the activity of ion channels.

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