

Application of FLIPR to the Evaluation of Mechanisms of Action of Novel Neurotoxins: Ion Channels as Molecular Targets

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A variety of marine neurotoxins produce autocrine excitotoxicity in the central nervous system (CNS). This neurotoxic action is mediated by endogenous glutamate which is released from glutamatergic neurons in the central nervous system. One such marine neurotoxin is domoic acid which is an amino acid analog of the excitatory amino acid transmitter glutamate. Domoate-induced neuronal injury is mediated primarily through NMDA receptors, that are activated secondarily as a consequence of AMPA/kainate receptor-mediated stimulation of glutamate release. Real time alterations in Fluo-3 loaded neuronal calcium concentrations were monitored using FLIPRT. The neurotoxic actions of domoate were associated with $[Ca^{2+}]$ influx through voltage-sensitive calcium channels, the reverse mode of operation of the sodium-calcium exchanger and the NMDA receptor.

Brevetoxins are potent lipid-soluble polyether compounds that are known to bind to and modulate voltage-gated sodium channels. These neurotoxins are produced by the marine dinoflagellate Karenia brevis. Brevetoxins produced acute neuronal injury and death in cerebellar granule cells. The brevetoxin-induced injury in cultured rat neurons was mediated by NMDA receptors that are activated indirectly as a consequence of brevetoxin-induced sodium channel activation with attendant release of glutamate. Similar to the results with domoate, brevetoxin neurotoxicity was associated with an increase in neuronal vulnerability being governed primarily by the NMDA receptor calcium influx pathway as revealed by pharmacologic experimentation using FLIPR.

Antillatoxin is a lipopeptide derived from the pantropical marine cyanobacterium, Lyngbya majuscula. Antillatoxin is neurotoxic in primary cultures of rat cerebellar granule cells and this neuronal death was prevented by both tetrodotoxin and NMDA receptor antagonists. Using Fluo-3 loaded neurons in FLIPR plates, the antillatoxin-induced calcium influx in cerebellar granule cells was shown to be antagonized by tetrodotoxin. Antillatoxin was found to be a novel activator of voltage-gated sodium channels. The neurotoxic actions of antillatoxin therefore resemble those of brevetoxins which produce neural insult through depolarization-evoked sodium load, glutamate release, relief of magnesium block of NMDA receptors, and calcium influx.