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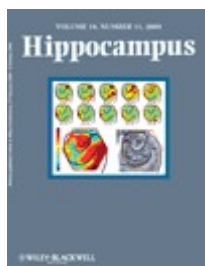
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Research Article

Phoneutria spider toxins block ischemia-induced glutamate release, neuronal death, and loss of neurotransmission in hippocampus

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calcium channels blockers • ischemia • neuroprotection

ABSTRACT

The aim of this study was to investigate the effect of spider toxins on brain injury induced by oxygen deprivation and low glucose (ODLG) insult on slices of rat hippocampus. After ODLG insult cell viability in hippocampal slices was assessed by confocal microscopy and epifluorescence using the live/dead kit containing calcein-AM and ethidium homodimer and CA1 population spike amplitude recording during stimulation of Schaffer collateral fibers. Spider toxins Tx3-3 or Tx3-4 and conus toxins, ω -conotoxin GVIA or ω -conotoxin MVIIC are calcium channel blockers and protected against neuronal damage in slices subjected to ODLG insult. Confocal imaging of CA1 region of rat hippocampal slices subject to ischemic insult treated with Tx3-3, Tx3-4, ω -conotoxin GVIA or ω -conotoxin MVIIC showed a decrease in cell death that amounted to $68 \pm 4.2\%$, $77 \pm 3.8\%$, $32 \pm 2.3\%$, and $46 \pm 2.9\%$, respectively. This neuroprotective effect of Tx3-4 was corroborated by electrophysiological recordings of population spikes amplitudes in CA1. The neuroprotection promoted on hippocampal slices by Tx3-3 or Tx3-4 was also observed when the toxins were applied 10, 20, 30, 60, 90, or 120 min after induction of the ODLG injury. During the ischemic insult, glutamate release from slices was increased by 71% (from 7.0 ± 0.3 nM/mg of protein control slices not subjected to ischemia to 12 ± 0.4 nM/mg of protein in slices exposed to ischemia). Tx3-3, Tx3-4, ω -conotoxin GVIA or ω -conotoxin MVIIC inhibited the ischemia-induced increase on glutamate release by 54, 72, 60, and 70%, respectively. Thus Tx3-3 and Tx3-4 provided robust ischemic neuroprotection showing potential as a novel class of agent that exerts neuroprotection in an in vitro model of brain ischemia. © 2009 Wiley-Liss, Inc.

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