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Activity:

Neuroprotection of hippocampal CA1 neurons from ischemic cell death using the calcium binding protein aequorin

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During ischemia, the deprivation of blood flow and oxygen to the brain results in excessive calcium influx through glutamate receptors, which can rapidly trigger cell death. One way neurons protect themselves from the toxic effects of calcium is to buffer the calcium with calcium binding proteins (CaBPs). Previous work has demonstrated that hippocampal neurons expressing the CaBP calbindin-D28k are better able to withstand an excitotoxic insult than neurons lacking calbindin. We have been investigating the feasibility of regulating calcium levels during ischemia by replenishing CaBPs. Aequorin (AQ) is a 22 kDa CaBP isolated from the coelenterate *Aequorea victoria*. AQ has been used for years as an auto-fluorescent indicator for monitoring calcium levels and has been shown to be safe and well tolerated by cells. The present studies were designed to test the hypothesis that intrahippocampal infusion of AQ can protect neurons from an ischemic insult. Rats were stereotaxically implanted with bilateral cannula (in the CA1 region of the dorsal hippocampus) under aseptic conditions. After recovery, rats received an intrahippocampal infusion of AQ (0.4%, 1%, or 4%) in one hemisphere and artificial CSF (aCSF) in the other (0.5 μ l/min for 1 min). Twenty-four or 72 hours following the infusion, coronal brain slices (400 μ m) were cut with a vibratome. Slices were maintained in oxygenated aCSF for 1 hr. They were then subjected to a 5-min oxygen-glucose deprivation (OGD), returned to oxygenated aCSF (with 0.2% trypan blue) for a 30-min reperfusion and then rinsed in oxygenated aCSF. All slice experiments were carried out at 35 °C. Slices were then fixed, cryoprotected, sub-sectioned (40 μ m), mounted, and coverslipped. An individual blind to treatment group counted the number of trypan blue stained (dead) CA1 neurons, and the number of dead cells in the AQ-treated hemisphere was compared to the aCSF-treated hemisphere to calculate a percent rescue. AQ treatment prior to OGD resulted in significantly fewer trypan blue stained CA1 neurons relative to control. In addition, the rats injected with 4% AQ had more rescue ($58 \pm 12\%$) than those injected with 0.4% AQ ($37 \pm 20\%$). However, when OGD was initiated 72 hours after 4% AQ infusion, no neuroprotection was noted. We are currently evaluating other time points to determine the time course over which AQ is neuroprotective. These data support the hypothesis that AQ may be an effective neurotherapeutic against ischemia when administered within 24 hours prior to an ischemic insult. We are also in the process of determining whether delivery of AQ is neuroprotective when administered following an ischemic insult.

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