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Effects of aequorin on cell death following ischemia. An experiment was carried out to investigate the potential benefit of the calcium binding protein aequorin on ischemia. Aequorin (4%) was dissolved in calcium-free cerebrospinal fluid with 6% DMSO (dimethyl sulfoxide). Two to three hours later, the rat was anesthetized with isoflurane and sacrificed by decapitation. Four hundred micron thick brain slices were prepared using a temperature-controlled Vibratome. Slices were immediately placed into oxygenated (95% O₂/5% CO₂) artificial cerebrospinal fluid (aCSF) of the following composition (in mM): 124 NaCl, 2.8 KCl, 2 MgSO₄, 2 NaH₂PO₄, 2 CaCl₂, 26 NaHCO₃, 0.4 sodium ascorbate, 10 D-glucose, pH 7.4, ~30 °C). After 1 hr recovery, slices were subjected to a 5 min ischemic episode. Ischemia was induced by replacing the glucose with fructose and replacing the oxygen with nitrogen. Following the 5 min ischemic challenge, slices were returned to normal oxygenated aCSF containing 0.04% trypan blue and incubated in the trypan blue aCSF for 30 min. The trypan blue exclusion method (healthy cells exclude trypan blue whereas dead or dying cells will take up the trypan blue and thus appear blue) is commonly used for evaluating cell death in cell culture or brain slices. After incubating in trypan blue, the sections were removed and placed in fixated overnight followed by 3 hr incubation in 30% sucrose. The 400 μm thick slices were then sectioned to a thickness of ~40 μm using a cryostat, mounted onto gelatin-coated slides and coverslipped. This preliminary experiment (see Figure 1) suggests that injection of aequorin directly into the hippocampus may confer neuroprotection against an ischemic insult.

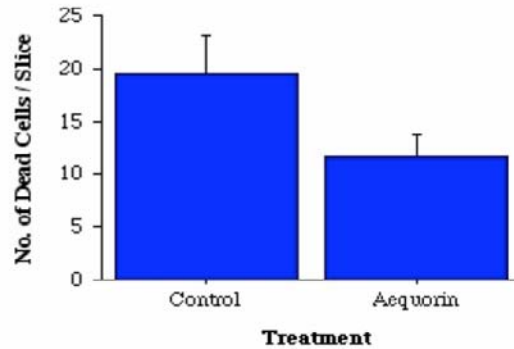
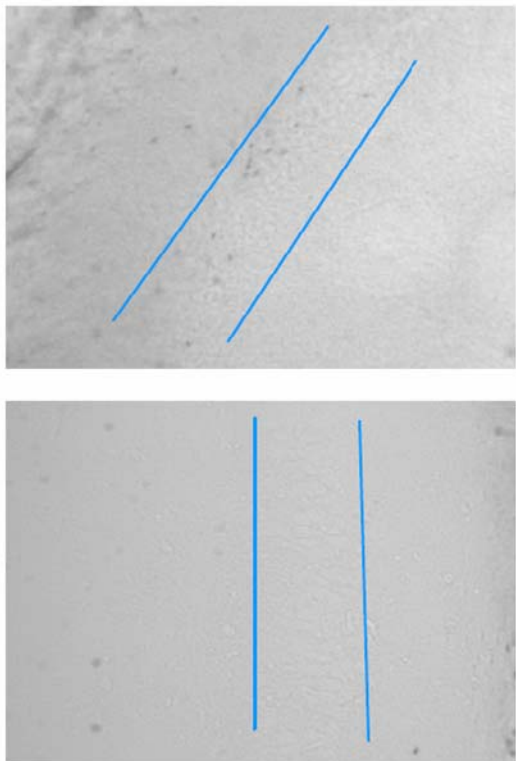


Figure 1. Aequorin may be neuroprotective when administered prior to ischemia. Two hours before an ischemic challenge, rats were injected with control CSF (left hemisphere) or aequorin (right hemisphere) directly into the hippocampus. After preparing brain slices, sections were subjected to an ischemic episode and dead cells stained with trypan blue. Left panels are video micrographs of hippocampus (CA1 region located between the solid lines). Notice that there are more trypan blue stained (dead) neurons in the control (upper left) relative to the aequorin (lower left) injected neurons. Right panel shows a bar graph of the mean number of trypan blue containing (dead) neurons after ischemia.

*This statement has not been evaluated by the FDA. This product is not intended to diagnose, treat, cure, or prevent any disease.
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