

DIFFERENTIAL EXPRESSION OF TRPM2 AND TRPV4 CHANNELS AND THEIR ROLE IN OXIDATIVE DAMAGE OF ORGANOTYPIC HIPPOCAMPAL CULTURES - A MICROSCOPIC PERSPECTIVE

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Both TRPM2 and TRPV4 channels of the TRP channel family are Ca^{2+} permeable and sensitive to excessive level of reactive oxygen species (TRPM2), or cell swelling, low pH and high temperature (TRPV4), factors that are often associated with various pathological brain damages including ischemia. These channels are known to be widely expressed in the brain but their exact expression pattern and function are not well understood. We aimed to investigate the cellular expression of these channels and their roles in oxidative stress-induced cell damage in organotypic hippocampal slice cultures (a model widely used in neurotoxicological studies of the hippocampus, a part of the brain highly vulnerable to damage in a number of neurological diseases). Channel expression was confirmed with RT-PCR and western blotting. Immunocytochemistry combined with both fluorescent and confocal microscopic analysis revealed the expression of TRPM2 in MAP2- positive CA1-CA3 pyramidal neurons, and TRPV4 in GFAP-positive astrocytes. When organotypic slice cultures were treated with exogenous H_2O_2 (600 μM), preferential damage was found in TRPM2-expressing pyramidal neurons but not TRPV4-expressing astrocytes, as assessed 24 hrs later using the uptake of fluorescent dye propidium iodide. By contrast, increased endogenous ROS production evoked with mercaptosuccinate (MCS; 400 μM) or buthionine sulfoximine (BSO; 4 μM) mainly damaged TRPV4-expressing hippocampal astrocytes, but not TRPM2-expressing pyramidal neurons. A decrease in the level of reduced glutathione was also associated with MCS and BSO-induced cell damage, as determined by a decrease of ThiolTracker fluorescence. Antioxidants (Trolox 500 μM ; MitoE 2 μM) reduced both neuronal and astrocytic cell death. We conclude that TRPM2 and TRPV4 proteins are differentially expressed in CA1/CA3 hippocampal pyramidal neurons and astrocytes, suggesting distinct pathophysiological roles of these channels in oxidative stress-induced cell damage, although the exact relationship between activation of these channel and cell death still remains to be determined.