Changes in ryanodine receptor expression and activity in hippocampal neurons exposed to BDNF or amyloid β peptide oligomers.

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Ryanodine receptors (RyR), by amplifying neuronal calcium entry signals via calcium-induced calcium release, participate in some types of synaptic plasticity and spatial memory formation. Postsynaptic RyR-mediated Ca^{2+}-induced Ca^{2+} release is required for late-LTP whereas presynaptic RyR mediate the secretion of brain derived neurotrophic factor (BDNF), a neurotrophin essential for some types of synaptic plasticity. Here, we studied RyR expression and activity in primary rat hippocampal neurons incubated for 6 h with 50 ng/ml BDNF. To inhibit RyR activity, neurons were pre-incubated for 1 h with 50 μM ryanodine and maintained with ryanodine during BDNF incubation. Neuronal RyR distribution was determined by immunocytochemistry, RyR2 and RyR3 mRNA and protein expression was assayed by RT-PCR and Western blots using specific primers and antibodies, and RyR-mediated Ca^{2+} release was determined by time series confocal microscopy in neurons preloaded with FLUO-4 AM. In mature hippocampal cultures, RyR2 and RyR3 were present in neurons and glial cells, and were clustered in small aggregates in the dendrites of pyramidal neurons. Incubation with BDNF increased significantly RyR2 and RyR3 mRNA and protein expression; RyR inhibition by pre-incubation with ryanodine completely abolished these stimulatory effects. In addition, incubation of neurons (6 to 24 h) with 500 nM amyloid beta peptide oligomers (ADDLs), which in these conditions are synaptotoxic, induced significant down regulation of RyR2 and RyR3 at the mRNA level. Neurons exposed to BDNF for 6 h exhibited significantly increased Ca^{2+} signals following addition of 0.5 mM 4-chloro-methyl cresol, a specific RyR agonist, and this effect was prevented by pre-incubation with ryanodine. Likewise, 1 h preincubation with 50 μM ryanodine significantly attenuated Ca^{+2} signals induced by addition of NMDA for 5 min. Taken together, our results strongly suggest that RyR-generated Ca^{2+} signals mediate BDNF-enhanced expression of RyR2 and RyR3 mRNA and protein. Thus, RyR presumably form part of the molecular mechanisms that underlie synaptic plasticity in hippocampal neurons and that are inhibited by ADDLs.

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