

Examining the Effects of Environmental Pollutants on Ryanodine Receptor Function

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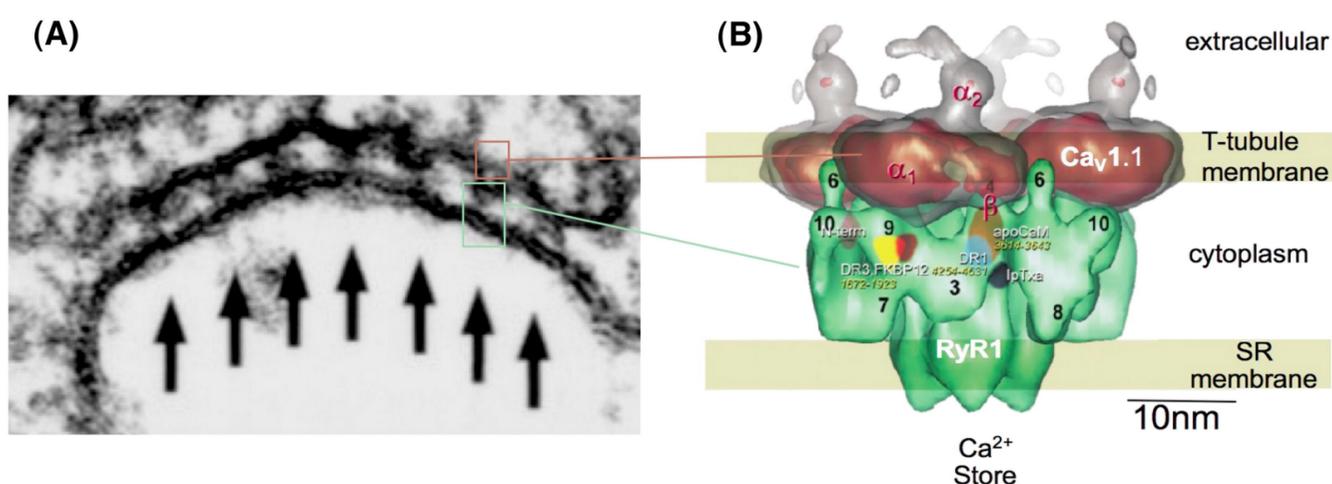
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Biology

Ryanodine receptors (RyRs) are a class of intracellular calcium channels present in neurons, cardiac muscle, and skeletal muscle which are thought to cause the release of calcium from the endoplasmic or sarcoplasmic reticulum upon activation by any one of several possible endogenous compounds. There is evidence to suggest that RyRs play a very important role in mediating a number of neurodevelopmental processes in both the central nervous system (CNS) and the peripheral nervous system (PNS), including modulating both chemical and structural aspects contributing to synaptic plasticity, and controlling both release of and response to neurotransmitters and neuropeptides via “sequential activation of [calmodulin] kinases, [cyclic adenosine monophosphate response element-binding] and transcription of genes encoding Ca²⁺-regulated proteins triggered by repetitive or prolonged depolarization of hippocampal neurons” (Pessah et al., 2010), among other influences. There are three isoforms of RyR, distributed in varying proportions in different tissues at different developmental stages. In adults, RyR1 is found mainly in skeletal muscle, RyR2 is expressed primarily in myocardium, RyR3 is predominant in the brain, and an additional, fiber-specific variant of RyR1 is thought to be expressed in some fish. Intriguingly, the distribution of the various RyR subtypes is altered significantly in early post-natal mammalian development, one of a multitude of factors which suggest that RyR function may play a role in the development of some cases of autism. In addition, hereditary early-onset Alzheimer’s disease (AD) has been found to be associated with genes encoding presenilin 1 or presenilin 2, both proteins have been found to regulate RyR expression and/or function, suggesting that functional RyR defects might also be implicated in the development of AD.

Numerous organic compounds are known or suspected of altering RyR activity. Two organic pollutants, Polychlorinated biphenyls (PCBs, a class of compounds which were widely used for industrial cooling and lubrication until the 1980s) and Triclosan (TCS, an antimicrobial agent commonly added to soaps and various other pharmaceutical and personal care products) have been shown to have a definite impact on calcium regulation via RyR activation in laboratory tests, and another pollutant, Bisphenol A (BPA, a component of many plastic products), is suspected of operating in a similar manner. It is speculation to say exactly what the implications of exposure to these chemicals might be in vivo or utero, but there is a potential link between the shocking increases in neurodevelopmental disorders over the last several decades and the increasing use of neurotoxic substances in agriculture, manufacturing, and personal care products.

While several studies have been conducted to examine the effects of PCBs and TCS on ryanodine receptor function, research investigating the potential impact of BPA on intracellular calcium concentrations is scarce. In addition, there seems to be little to no research which examines the effect of exposure to more than one of these compounds at one time, as it is presumed might often be the case in actual exposure scenarios. I aim to investigate what these effects might be. Having examined methods used by researchers who performed similar analyses, my experimental design currently includes culturing skeletal muscle cells which express one or more of the ryanodine receptor proteins and exposing these cells to set concentrations of one or more PCB congeners, BPA, and TCS separately and in various permutations to examine selected consequences of additive exposure and possible interaction effects, using a fluorescent calcium assay along with a known RyR agonist (such as caffeine) as a positive control and a known RyR antagonist (such as ruthenium red) as a negative control.



“(A) Electron micrograph of the T-tubule/SR junction of negatively stained skeletal myotubes. Arrows indicate the position of densely staining “junctional feet” that are the large cytoplasmic domain of a row of RyR1s that span the junctional space between the two membranes (adapted from (Protasi et al., 1998)). (B) 3-D model of the relative orientation of four CaV1.1 (i.e., α1s) L-type Ca²⁺ channel subunits (brown) and RyR1 (green) based on cryoEM reconstruction studies (adapted from (Wolf et al., 2003)).”