P003 Bcl-2 targeting ryanodine receptors: more than apoptosis?

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Anti-apoptotic B-cell lymphoma 2 (Bcl-2) proteins counteract apoptosis at the mitochondria by scaffolding pro-apoptotic Bcl-2-family members, but also act at the endoplasmic reticulum, controlling intracellular Ca^{2+} signalling. Bcl-2 suppresses Ca^{2+} release by targeting the inositol 1,4,5-trisphosphate receptor (IP_3R). The Bcl-2-binding site on the IP_3R shows striking similarities to a site present in all ryanodine receptor (RyR) isoforms. We now show that Bcl-2 interacts with RyRs in ectopic expression systems and in rat hippocampus. Detailed molecular studies (including SPR) revealed that Bcl-2, via its BH4, binds to purified RyR domains containing the putative binding site. Bcl-2 overexpression inhibited caffeine-induced Ca^{2+} release in RyR-expressing HEK293 cells. Consistent with the ability of the biotinylated BH4 domain to bind RyRs, a BH4-Bcl-2 peptide was sufficient to suppress RyR-mediated Ca^{2+} release in HEK293 cells and dissociated rat hippocampal neurons. Hence, these data indicate that besides IP_3Rs Bcl-2 targets RyR channels. Yet, while the BH4 domain of Bcl-XL fails to bind to and inhibit IP_3Rs, due to a critical conserved amino acid difference with BH4-Bcl-2 (i.e. Asp11 in Bcl-XL versus Lys17 in Bcl-2), BH4-Bcl-XL could target RyR channels, indicating that the binding determinants for complex formation with Bcl-2/Bcl-XL are similar for IP3Rs and RyRs, but not identical. These data now set the stage for discovering novel biological functions for anti-apoptotic Bcl-2 proteins by targeting RyR channels in different cell types.