ROLE OF AUTOPHAGY IN ISCHEMIA/REPERFUSION

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We used fluorescence microscopy to investigate the relationship between apoptotic and autophagic signaling pathways in HL-1 cardiomyocytes subjected to simulated ischemia/reperfusion (sI/R). Apoptosis was indicated by clustering of GFP-Bax at mitochondria. The dynamics and role of autophagy during sI/R were monitored using GFP-LC3. Autophagic flux was null during the ischemic period, and increased at reperfusion, but not to the same degree as under normoxic conditions. Double-labeling cells with mito-DsRed revealed that mitochondria were targets of autophagy, a phenomenon that was attenuated if cells were treated with cyclosporin A to prevent mitochondrial permeability transition pore opening. Enhancing autophagy using rapamycin or by overexpression of Beclin1 reduced sI/R activation of GFP-Bax. Inhibiting autophagy by 3-MA, wortmannin, RNAi knockdown of Beclin1 or expression of a dominant negative mutant of Atg5, a component of the autophagosomal machinery downstream of Beclin1, increased Bax activation following sI/R. Expression of Beclin1 lacking the Bcl-2 binding domain (Beclin1ΔBcl2BD) significantly reduced autophagic flux, and did not protect against sI/R injury, implicating Bcl-2 as a co-factor in the autophagic response. In apparent contrast, overexpression of Bcl-2 slightly suppressed autophagic flux; an effect enhanced by targeting Bcl-2 to the sarco/endoplasmic reticulum (S/ER). Suppression of autophagy by S/ER-targeted Bcl-2 may be in part due to depletion of S/ER calcium stores: intracellular scavenging of calcium by BAPTA-AM and treatment with thapsigargin, an inhibitor of the S/ER calcium ATPase (SERCA), significantly reduced autophagic activity in response to nutrient deprivation or sI/R. These findings reveal that Bcl-2 regulates the autophagic response at the level of S/ER calcium content rather than via direct interaction with Beclin1. Moreover, calcium homeostasis was identified as an essential component of the autophagic response in the cardiomyocyte. Residual levels of autophagy functioned to preserve cell viability following sI/R, and enhancing autophagy constituted a powerful and previously uncharacterized protective mechanism against I/R injury in the cardiac myocyte.