INTRODUCTION: Jun Kinase (JNK) is an important regulator of apoptosis during cellular stress, but also has pro-survival properties in some cell types. JNK has been shown to protect cardiomyocytes against nitrosative and oxidative stress-induced apoptosis\textsuperscript{1,2}; however, mechanisms of cytoprotection are not understood. We used a model of H$_2$O$_2$-induced cardiac myocyte apoptosis in culture to study the role of JNK in this process.

METHODS: Primary cultures of rat neonatal cardiomyocytes were grown as previously described\textsuperscript{1,2}. We constructed adenoviruses expressing dominant negative Jun kinase (AdDNJ) or inactive fusion protein JNKK2-JNK1(APY) to inhibit endogenous Jun kinase (JNK) activity. Apoptosis was induced by hydrogen peroxide (H$_2$O$_2$, 200 $\mu$M) and caspase-9 and caspase-3 activities were monitored using a colorimetric assay with or without JNK inhibition. In vitro caspase-9 and caspase-3 cleavage was also performed using cytosolic lysates with or without addition of cytochrome c/ATP. Coimmunoprecipitation was used to determine whether JNK interacts with APAF1, an adaptor protein important for the type II caspase-dependent cell death pathway.

RESULTS: Because oxidant stress activates mitochondria-dependent apoptosis, we examined the effect of JNK inhibition on various steps in this pathway. Cytochrome c release in myocytes was unaffected by JNK inhibition, but caspase-9 and caspase-3 activation were accelerated (peak at 8 hour compare to 12 hour in control, p<0.05). AdDNJnk also increased the cytochrome c/ATP-mediated cleavage of caspase-9 and caspase-3 in cell-free extracts of cardiac myocytes, but not of HEK293 cells, suggesting the presence of tissue-specific factors. JNK did not appear to interact directly with caspase 9, but reciprocal co-immunoprecipitation showed interaction of endogenous JNK with APAF1, a cofactor necessary for caspase-9 activation. IP-Western analysis showed that APAF-1 was heavily serine-phosphorylated under basal conditions in cardiac myocytes. JNK interaction was not dependent on APAF-1 phosphorylation, and APAF-1 phosphorylation was not directly related to the level of JNK activity, suggesting that it may not be a phosphorylation target for JNK.

DISCUSSION: JNK has been implicated in the regulation of mitochondria-mediated apoptosis through phosphorylation of Bcl-2 family members including Bad, Bcl2, BclXL and Mcl-1. It has also been suggested that caspase 9 may be a substrate for phosphorylation by ERK1/2, another member of the MAPK family. However, JNK has not previously been shown to have post-mitochondrial targets. Our study suggests that in some cell types JNK may inhibit caspase-9 activation and delay apoptosis by interacting
with APAF1. The significance of APAF1 phosphorylation and dephosphorylation, however, is still not known. Further studies will determine whether dephosphorylation of APAF1 affects APAF1 stability and its ability to form an active apoptosome complex.

ACKNOWLEDGEMENTS. This work was supported by a Grant-In-Aid from the American Heart Association (N.H.B.) and by a grant from the Miami Heart Research Institute (N.H.B.). Thanh Tran is the recipient of a Pre-Doctoral Fellowship Award from the American Heart Association (Florida/Puerto Rico Affiliate.)

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