SUMMARY AND CONCLUSIONS
Low to moderate doses (<10 μM) of NE induce hypertrophy in cardiac myocytes while higher doses (>50 μM) induce apoptosis. Although the mechanism of induction of hypertrophy is better understood, that of apoptosis is largely unknown and is of high clinical relevance. The present study involves investigating various aspects of the apoptotic process with primary emphasis on the transcriptional aspects. Although execution of apoptosis is the systematic dismantling of the cell structure, its induction involves modulation of a number of pro- and anti-apoptotic genes.

Cardiac myoblast cell line H9c2 has often been used as a surrogate for primary myocytes in investigating both hypertrophy and apoptosis. It responds to a variety of other stimuli like Ang II, NE, ATP and phorbol ester, thereby, demonstrating its close resemblance to cardiac myocytes. It also expresses the repertoire of α and β-adrenergic receptors similar to primary myocytes and hence, is a good model system for understanding the adrenergic events.

Upon treatment with a lower dose of NE (~2-10 μM) H9c2 cells induced proliferation (equivalent to hypertrophy for primary myocytes) while further increase in NE concentration to (50 -200 μM) induced apoptosis as evident from multitude of pro-apoptotic events such as Caspase-3 and PARP cleavage, internucleosomal DNA degradation, decrease in Bcl-2 and increase in Bax levels.

Thereafter, a prevailing hypothesis that a general increase in the intracellular reactive oxygen species (ROS) (by NE and other agonists like H2O2) is the key determinant of transition from hypertrophy to apoptosis was tested. H9c2 myoblasts upon treatment with 2 μM and 100 μM NE induced intracellular ROS only transiently and at a comparable level while 200 μM H2O2 treatment caused a robust induction of ROS. Analysis of a number of redox responsive transcription factors viz, NF-κB, AP-1 and Nrf2 as the downstream target of ROS signaling revealed that none of the agonists were effective in inducing NF-κB while the extent of induction of AP-1 was a direct function of ROS levels. Moreover, while 2 μM NE was only a moderate inducer of Nrf2, both 100 μM NE and 200 μM H2O2 induced it at a higher but comparable level, thereby, indicating interplay of multiple signal in puts in modulating downstream transcriptional regulators.
Further analysis of subsequent events revealed the induction of iNOS as a candidate mediator of apoptotic event further downstream (presumably by the generation of NO). Finally, it was demonstrated NE induce cell death by a mechanisms distinct from that by H2O2 and therefore presumably involve discrete signaling rather than a general surge of ROS.

Analysis of the status of FKHR, a prototype member of the FOXO family and a prime inducer of pro-apoptotic genes, also revealed a complex sequence of events till 24 hours post-NE exposure. There was an initial increase in FKHR level that was primarily (but not exclusively) nuclear localized and it remained in equilibrium (~ six hours) between the phosphorylated and non-phophorylated forms. Afterwards, the non-phosphorylated form prevailed till 24 hours following which it was degraded (well before the onset of apoptosis). Notably, although FKHR remained nuclear localized for an extended period, it was active in transcribing the cognate promoter reporter system only at the beginning and JNK (but not Akt) contributed to its activity. The relevance of induction of FKHR was further evident from its induction in the myocardium of rats injected with NE. Although the relevance of induction of FKHR, its modulation by phosphorylation-dephosphorylation and its transient transcriptional activity is yet to be understood, it nevertheless highlights a pertinent role of FKHR in the downstream events following NE exposure.

Insulin, a pro-survival growth factor, acts by sequestering FKHR in the cytoplasm (by its phosphorylation). Paradoxically, when added in conjunction with NE, insulin expedites the nuclear exclusion of FKHR but did not protect cell from apoptosis. Finally, over expression of FKHR was ineffective but that of a dominant active FKHR induced extensive cell death. Taken together, it appears that FKHR has a pro-apoptotic function (in NE treated cell) in H9c2 cells but it works not in isolation but in conjunction with other regulatory molecules by a mechanism yet to be understood.

Heart failure in conjunction with insulin resistance is the primary cause of morbidity in the industrialized society. Although advance drug regime has significantly contributed to the effective management of such conditions, the
biochemical basis of cell death in the failing myocardium is a prerequisite for the basic understanding of the mechanism.

The present study was primarily aimed at understanding the sequence of events (both biochemical and molecular) that leads to cell death in H9c2 myoblasts (as a surrogate for primary myocytes) upon exposure to NE. A systematic analysis has revealed that immediately after NE treatment, a number of events like reactive oxygen species generation and induction of transcriptional regulators like AP-1 and Nrf2 were documented. Although the immediate targets of those gene regulatory proteins and that of the reactive oxygen species are not known yet, this data nonetheless, provides an important lead to explore for those targets. It is noteworthy that in a parallel study exclusively on the role of AP-1 family members has already revealed a distinct pattern of induction of two Fos family members i.e., Fra-1 and FosB and also has assigned a distinct pro-survival role for Jun. (PhD thesis Neelakantan T.V.).

This study has also revealed a distinct modulation of FKHR, a member of the forkhead family of transcription factor, with specific reference to insulin. In view of close relationship between insulin resistance and heart failure, it will be of future interest to further investigate the precise role of FKHR in mediating the effects of NE as a down stream effector.

Finally, the study does not include the events that occur after 24 hours (immediately before the onset of apoptosis) barring the observation that iNOS is induced at later thereby, indicative of role of reactive nitrogen species in the apoptotic process.