Aims and Objectives
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Under pathological conditions demanding augmented cardiac performance, myocytes (terminally differentiated) undergo reprogramming of gene expression leading to hypertrophy. Physiological modulators of cardiac functions such as catecholamines, endothelin-1, angiotensin II also mimic such response(s) in neonatal and adult myocytes cultured ex vivo (Simpson, 1985; Clerk A, 2002; Sadoshima and Izumo, 1995). Paradoxically, at least some of these agonists (e.g., angiotensin II and norepinephrine) also cause programmed myocytes loss (apoptosis) at higher doses (Ph.D thesis, Manveen K Gupta, Special Centre for Molecular Medicine, JNU, 2005). Although these two processes have generally been investigated as independent phenomena and the ultimate fate of a myocyte is distinctive (either hypertrophy or apoptosis), numerous studies also indicate that the cognate pathways are mutually interactive rather than exclusive. For example, while a number of signal transducing kinases (e.g. S6, Ras, Raf, ERK, p38), phosphatases (e.g. calcineurin) and down-stream transcriptional regulators (e.g. NFAT, GATA-4, AP-1, NF-kB and MEF-2) have been identified as the mediators of hypertrophic responses, molecules such as ERK and JNK have also been implicated in mediating anti- and pro-apoptotic responses (Wong et al., 1998; McKinsey et al., 2000; Frey and Olson, 2003; Nadrutz et al., 2004; Pikkarainen et al., 2004; Baines and Molkentin, 2005). It remains to be explored as to how these pathways interact with each other and eventually lead to very distinct biological outcome.

Cardiac myocytes upon exposure to various pro- and anti-hypertrophic as well as pro- and anti-apoptotic stimuli elicits an immediate early response characterized by the activation of AP-1. Also diverese stimuli, xenobiotic and oxidative stress, UV irradiation, bacterial and viral infections, growth factors and cytokines have been reported to induce AP-1 in various cell types. Biochemical mechanisms by which such divergent stimuli integrate to this common mediator of gene expression and still achieve disparate downstream consequences have been a subject of immense interest (Clerk A, 2002; Hazzalin and Mahadevan, 2002; Fleegal and Sumners, 2003; Frantz et al., 2003; Hess et al., 2004; Nadrutz et al., 2004; Taimor et al., 2004).
AP-1 activities consist of heterodimers of Jun and Fos proteins viz., Jun, JunB, JunD and Fos, FosB, Fra-1, Fra2. They dimerize through a conserved sequence motif called leucine zipper, and bind to the canonical DNA sequence TGAG/CTCA (TRE). Fos proteins can not dimerize to each others, while the Jun proteins can do so but the complex is unstable and presumably do not exist in vivo. Jun-Fos proteins are the prototypical members of an even larger family of gene regulatory proteins classified as basic leucine zipper (b-Zip) family. Notably, Jun can also dimerize with certain other members of the family such as CREB, Maf and ATF-2 and bind to DNA sequences similar to TRE, thereby further extending the repertoire of functional AP-1 dimers.

Gene knockout, forced expression, mRNA depletion and dominant negative mutational analyses of different Jun-Fos proteins have assigned distinct functions for each member. However, it is not understood as yet how different Jun-Fos dimers, apparently bind to the same target sequence (TRE) still differentiate between their target genes. Recent studies have also indicated that various Jun-Fos proteins are induced by distinctive signaling cascades although the details of which is yet to be discerned.

AP-1 has long been attributed to cardiac hypertrophy in general and to the induction of certain markers such as ANF and α-skeletal actin in particular. However, the precise role of AP-1 in reprogramming of gene expression under various pathophysiological conditions is yet to be deciphered. Moreover, AP-1 is also induced (in cardiac myocytes) by various apoptotic stimuli but the precise role it plays in inducing (or preventing) apoptosis is completely unknown.

In the present study, I have used norepinephrine (NE) as an agonist for inducing hypertrophy (at 2 μM concentration) and apoptosis (at 100 μM concentration) in H9c2 cardiac myblasts and analyzed AP-1 as a paradigm to understand how cardiac myocytes processes various signal in puts and lead to those distinctive consequences. Over the past decade H9c2 cells have often been used as a faithful surrogate for investigating mechanisms of cardiac myocyte hypertrophy and apoptosis (Chen et.al., 2000; Huang et.al., 2004; Ihara et al., 2005; Planavila et al., 2005).
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The first objective of this study was to test the premise that AP-1 activities induced under two conditions are qualitatively and quantitatively different. This possibility was tested by:

a) Analyzing the kinetic of induction of AP-1 (DNA binding) by low and high doses of NE (and also by a number of other agonists).

b) Analysing the transcriptional activation of AP-1 by low and high doses of NE.

c) Analyzing the constituents of AP-1 complexes induced in H9c2 myoblasts (by 2μM and 100μM NE) by using specific antibodies against various Jun and Fos proteins.

d) Finally confirmation of results (of induction of various Jun/Fos proteins) in vivo in rat myocardium.

The second objective of this study is to analyse the biochemical mechanisms by which various Jun and Fos proteins are differentially expressed under two conditions of NE stimulation (2 μM and 100 μM respectively). Towards that objective:

a) The kinetics of induction of MAPKinases (ERK, p38 and JNK) and PI3kinase pathways by 2 μM and 100 μM NE will be analyzed.

b) Thereafter, the contribution(s) of each of these kinases towards the induction of AP-1 activities and that of members differentially expressed under hypertrophy and apoptosis respectively will be studied.

Finally, the role of the selected AP-1 members in myocyte apoptosis (and survival) will be determined by

a) Over expression of selected Jun/Fos proteins.

b) Attenuation of Jun activity by a dominant negative mutant.

c) Attenuation of activity of selected Jun/Fos proteins by antisense oligonucleotides.