Phosphorylation c-Jun and ATF2 in ventricular myocytes by endothelin and phenylephrine.

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The cardiac myocyte is a terminally differentiated cell. In response to increased myocardial workload or damage, myocytes undergo hypertrophic (as opposed to hyperplastic) growth. This response includes transient up-regulation of immediate early genes such as c-jun [1,2]. Expression of c-jun is partly controlled by c-Jun/ATF2 dimers bound to AP-1-like sites in the c-jun promoter [3,4]. Phosphorylation of c-Jun and ATF2 by stress-activated protein kinases (SAPKs) increases their transactivating activities [5-8]. The hypertrophic response in vivo can be simulated in myocytes cultured from neonatal rat heart ventricles exposed to agonists such as the α-adrenergic agonist phenylephrine (PE) or the vasoconstrictor peptide endothelin-1 (ET-1) [2], which activate the extracellular-regulated kinases (ERKs) and, to a lesser extent, the SAPKs [9-12]. We have studied the effects of ET-1 and PE on the induction and phosphorylation of c-Jun and ATF2 in cultured ventricular myocytes using Western blot analysis.

Ventricular myocytes, isolated and cultured by standard methods [11,12], were stimulated with ET-1 (0.1 μM) or PE (50 μM). Nuclear extracts were prepared [13] and proteins (100 μg) were separated by SDS-PAGE using 10% and 8% acrylamide gels to study c-Jun and ATF2 respectively. After transfer to nitrocellulose, specific proteins were identified using primary antibodies to c-Jun and ATF2 (Santa Cruz Biotechnology) and horseradish peroxidase-linked secondary antibodies. Bands were detected by the ECL method. Phosphorylated forms of c-Jun and ATF2 were identified as reduced mobility bands.

ET-1 stimulated a small apparent increase in total c-Jun protein over 30 min (Fig. 1). The protein synthesis inhibitor, cycloheximide (20 μM) did not inhibit this increase. This inhibitor itself activated the SAPKs and induced an increase in c-Jun immunoreactivity. The apparent increase in c-Jun probably represents a change in its phosphorylation state as is seen in cell stress [13]. ET-1 stimulated a second phase of increase in total c-Jun immunoreactivity. This was maximal at approximately 1 h and remained above control levels over 6 h (Figs. 1 and 2). The second phase was inhibited by cycloheximide (Fig. 1), indicating that it represents de novo biosynthesis of c-Jun protein. PE induced c-Jun immunoreactivity but to a lesser degree than ET-1. In comparison with ET-1, the time course was delayed, reaching a maximum at 1.5 - 2 h (Fig. 2). Inhibition of protein kinase C with 10 μM Ro 31-8220 abolished the second phase of c-Jun induction by ET-1 (Fig. 1) and PE. In addition to increasing total c-Jun immunoreactivity, ET-1 induced the appearance of phosphorylated c-Jun, identified as a reduced mobility band of approximately 39 kDa apparent molecular mass (Figs. 1 and 2). Low levels of phosphorylated ATF2 (reduced mobility bands of approximately 76 kDa apparent molecular weight) were also detected (data not shown). PE induced phospho-c-Jun to a lesser degree than ET-1, and had little effect on ATF2 phosphorylation. In summary, the hypertrophic agents, ET-1 and PE, induced the synthesis and phosphorylation of c-Jun in ventricular myocytes. ET-1 also stimulated the phosphorylation of ATF2. These changes may be involved in transcriptional up-regulation during cardiac hypertrophy.