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Apoptotic cardiomyocyte hypertrophy during sepsis and septic shock results from prolonged exposure to endothelin precursor

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1. ABSTRACT

Septic shock is a complex cardiovascular dysfunction which leads to regional circulatory alterations and multi-organ dysfunction in humans and animal models. To elucidate the role of stress-activated signaling molecules in the regulation of myocardial dysfunction, we have developed and standardized isolated ventricular myocyte techniques. These techniques allow the assessment of cardiodynamics at cellular (ventricular myocyte) level. These studies are carried out in a well defined model of systemic inflammatory response syndrome following polymicrobial sepsis in the rat. Evidence is provided that sepsis-induced myocardial dysfunction produces indications (signs) of early stages of heart failure. This evidence correlates with upregulation of stress-activated protein kinase cascade. These findings suggest that prolonged exposure to endothelin precursor causes decompensatory hypertrophy in adult rat ventricular myocytes (ARVMs) during sepsis. The decompensatory hypertrophy could, in turn, results in increased cytosolic caspases-3 activity in ARVMs.

2. INTRODUCTION

Sepsis is an acute circulatory dysfunction which arises as sequelae to a serious, usually systemic infection. The mortality from septic shock syndrome ranges from 20-90%, depending upon the patient's age and associated pathologies (1). The incidence of sepsis has increased during the last 20 years, with more than 500,000-1 million patients developing sepsis each year in the US (2). According to National Vital Statistics Report (2004), septicemia and sepsis are now the 10th leading cause of death in the US, as opposed to being the 13th leading cause of death in 1990 (3). Sepsis-induced multi-organ failure is a well-known entity. Despite the fact that a number of septic patients exhibit heart dysfunction, it is not considered a major threat in ICUs. It is for this reason that pharmacotherapeutic options and the cellular pathophysiological mechanisms underlying sepsis-induced myocardial dysfunction have not been studied extensively.

2.1. Sepsis-induced cardiomyocyte dysfunction

Sepsis produces myocardial dysfunction in both animals and humans (4-8). In animal models, sepsis

showed an increase in cardiac output and vascular resistance during the hyperdynamic stage of sepsis. It was observed that chronic peritoneal sepsis in the rat depressed the rates of left ventricular contraction and relaxation in an isolated heart preparation (7). ARVMs isolated from septic rat heart exhibited a depression in peak shortening, along with reduced rates of shortening and relengthening (+dL/dt and -dL/dt, respectively), suggesting substantial contractile dysfunction (8). Septic ARVMs were also found unresponsive to an increase in extracellular calcium (0.5-3 mM) (8). Interestingly, although septic ARVM (isolated from 24h post sepsis rat heart) exhibited depressed contractile response at 3h post-incubation in culture media, the contractility was found to be elevated at 24h as evident by increased PS, +dL/dT and - dL/dT (9).

The findings from our laboratory suggest that both isolated heart preparation and ARVMs produce similar functional and mechanical responses following sepsis. Recent data from our laboratory suggested that during a hyperdynamic stage, sepsis caused an increase in ventricular filling pressure (left ventricular end diastolic pressure, LVEDP) and time constant for left ventricular relaxation (*tau*) (10). We also observed that progression of sepsis from 1-day to 7-days produced deterioration of left ventricular performance and exhibited changes in the rates of left ventricular relaxation, -dP/dt, and contraction, +dP/dt (10). These observations provide evidence for early stages of heart failure but were quite distinct from classical tachycardia-induced heart failure. The rapid progression of heart dysfunction during sepsis-induced tachycardia could be due to a cumulative physiological response to sympathetic stimulation, elevated levels of cytokines, endothelin-1 (ET-1) and activation of signaling molecules at the cellular level.

3. ENDOTHELIN MECHANISMS IN CARDIOMYOCYTES

Endothelin isopeptides, ET-1, ET-2 and ET-3 regulate a variety of biological effects in non-vascular tissues (11, 12). In cardiac myocytes, they exert a positive inotropic and negative lusitropic effect along with pressure overload-induced cardiac hypertrophy (13,14). ET-1 levels have been found to be elevated in various pathophysiological states such as chronic heart failure (CHF) (15). During severe CHF in humans, an elevation in the plasma ET-1 levels principally represents an elevation in the bigET-1, a precursor of ET-1 (16). However, the role of elevated ET-1 levels during sepsis and septic shock remains to be elucidated. Endothelin isoforms also regulate the movement of the intracellular concentrations of the ions, thus affecting the contractile properties of the cardiac myocytes (13).

ET-1 acts via a large transmembrane receptor family known as guanine nucleotide-binding protein-coupled receptors (GPCRs) (17). ET-1 exerts its effect in mammals through two GPCR-receptor subtypes namely, ET_A and ET_B (18). The ET_A receptor is present in the neonatal rat cardiac myocytes (19). ET-1 via ET_A receptor stimulates the formation of inositol 1, 4, 5- triphosphate and diacylglycerol, which is detectable within seconds in cardiomyocytes (20). Diacylgylcerol which remains in the membrane causes the translocation of the δ-and ε-isoforms of protein kinase C (PKC) to the compartment, resulting in PKC activation (21). Phospholipase D which instantly hydrolyzes phosphatidate to DAG also provides an alternative pathway for the DAG formation and is stimulated by myocytes in the presence of ET-1 (22,23).

ET-1 also exerts its effects through the regulation of the ion movement, such as Ca^{2+}ions in cardiac myocytes. Endothelin isopeptides exhibit their positive inotropic effect through an increase in the $Ca^{2+}{}_i$ transients in cardiac myocytes. The changes in the Ca^{2+} movements induced by ET-1 are thought to be produced by the stimulation of Na^+/H^+ exchanger I and sarcolemmal L-type Ca^{2+} channel (13). The next events involve the activation of small G-protein Ras and extracellular signal regulated kinase ½ (ERK1/2), c-Jun N-terminal kinase and p38-mitogen – activated protein kinase (p38-MAPK) cascades (21). Small molecules including protein kinases (90-kDa ribosomal protein S6 kinase, MAPK-activated protein kinase 2), nuclear transcription factors (GATA-4, c-Jun) are also responsible for the overall biological effect of ET isopeptides on the cardiac myocytes (24-26).

4. SIGNALING MECHANISMS IN CARDIOMYOCYTES

Mitogen-activated protein kinase (MAPK) signal transduction pathways play a crucial role in the regulation of eukaryotic cells. Currently, there are five members in the MAPK family which have been characterized in mammals: extracellular signal-regulated kinase ½ (ERK1/2), p38-MAPK, c-Jun N-terminal kinase (JNK), big MAPK1 (ERK5), and ERKs 3 and 4 (27, 28). However, ERK1/2, JNK and P38-MAPK have been extensively studied. In the current review, we will be discussing the role of these signaling kinases in cardiac myocytes during sepsis.

4.1. p38-MAPK

Activation of p38- mitogen activated protein kinase (MAPK) signaling cascade is one of the mechanisms by which cells respond to the extracellular stimuli like the stress, UV, proinflammatory cytokines and endotoxin (27, 29). The p38-MAPK cascade consists of MAPKKKs: MEKK1-4, MLK2 and -3, DLK, ASK1, Tpl2, and Tak1; MAPKKs: MEK3 (MKK3), and MEK5 (MKK6); MAPK: p-38 MAPKs. The mammalian p38- MAPK is homologous to HOG1, the osmosensing MAPK of *S. cerevisiae* (30).

Several isoforms of p38-MAPK have been characterized: alpha-1/alpha-2, beta-1/beta-2, gamma, and delta (31-35). The isoform of p38-MAPK delta is expressed widely in various adult tissues and during development while p38-MAPK gamma isoform is predominantly expressed in skeletal muscle. The alpha and the beta isoforms of p38-MAPK isoforms are more prevalent in the human heart than the two other isoforms (33). The dual phosphorylation of Thr180 and Tyr182 by the upstream MAPKK, MKK6 and MKK3 activate p38-

MAPK (33). MKK3 and MKK6 are in turn activated by several MAPKKKs in response to stress stimuli (36). MKK3 and -6 are highly specific for p38-MAPK activation (37-43). There is another suggestive mechanism for the activation of p38-MAPK which is independent of the prototypic MAPKKs cascade (44). Interaction of p38-alpha with TAB1 (transforming growth factor beta protein kinase 1 (TAK1)-binding protein 1) produces intramolecular autophosphorylation and activation of p38-alpha. TAB1 is not a MKK and has no catalytic activity but appears to be an adaptor or a scaffolding protein (44). ET-1 is also known to activate p38-MAPK cascade in cardiac myocytes (45, 46). The activation of p38-MAPK has been observed during ischemia/reperfusion, Oxidative stress and heart failure in both human and animal models (47-51). It has also been implicated in hypertrophy of cardiac myocytes (52, 53). However, inhibition of p38-MAPK activity in vivo attenuates heart failure and cardiac remodeling during myocardial infarction (54). The biological role of p38-MAPK is probably pro-apoptotic, however, its role in cardiac myocytes as cytoprotective, hypertrophic or pro-apoptotic remains to be elucidated (48, 55, 56).

4.2. ERK

Extracellular signal-regulated kinases, ERK1 and ERK2 were initially identified as 'microtubule-associated protein kinases' but later recognized as 'mitogen activated protein kinases' as they were activated by mitogens (21). ERK family of proteins are implicated in survival signaling in cardiac myocytes. It has also been postulated to be a protective signaling mechanism during apoptosis (57). The ERK1/2 cascade gets activated by dual phosphorylation of a Thr- and a Tyr-residue in a Thr-Glu-Tyr motif (58). The activation of the ERK1/2 cascade in terms of biological response is considered to be anabolic (55). In the heart, it stimulates the growth and promotes adaptive hypertrophy (55). The ERK1/2 cascade has been described in which the two serine residues in MAPK kinases 1 and 2 (MKK1 and 2, MEK1 and 2) are phosphorylated and activated by the MAPK kinase kinase Raf. MKK1/2 then subsequently phosphorylates and activates ERK1/2. All the three stages in the ERK 1/2 cascade, namely, c-Raf and A-Raf, MKK ½ and ERK1/2 are rapid (3-5 min) and powerfully activated by ET-1 in cardiac myocytes (59, 60). The activation/ phosphorylation induced by ET-1 in ERK1/2 cascade is stoichiometric and similar to that produced by PMA (21). The ERK1/2 cascade plays an important role in the regulation of transcription and growth in cardiac myocytes. During transcription factor phosphorylation, the ERK1/2 cascade appears rapidly in the nucleus of the cardiac myocytes (61).

4.3. JNK

JNK or c-Jun N-terminal kinases, one of the member of MAPK superfamily are activated by Thr-Xaa-Tyr motifs (27). JNKs, also referred as stress-activated protein kinases, are potently activated by stress, UV, proinflammatory cytokines, DNA-damaging agents, and growth factors, etc (27). Mammalian JNKs encoded by three separate genes as JNK1, JNK2 and JNK3 are located on chromosomes 10q11.1-11.2, 5q35.3, and 4q21-g22.1, respectively (62). JNKs are activated by stress, DNA-damaging agents, radiation, pro-inflammatory cytokines and growth factors. The review article by Sugden, 2003 provides an insight into the regulation and expression of c-Jun activity. The c-Jun binds to atleast two consensus sequences which are frequently found in the promoter region of the genes (21). It binds preferentially as a heterodimer along with the c-Fos transcription factor to the AP-1 site (TGAGTCA) and to the CRE site (TGAGCTCA) preferentially as a heterodimer along with ATF-2 transcription factor. The phosphorylation of two Ser-residues (Ser-63 and Ser-73) within the N-terminal domain stimulates the transactivating activity of c-Jun (63). The function of other two sites for phosphorylation (Thr-91 and Thr-93) in the N-terminal part of the protein is not clear (64).

The expression of c-Jun protein and c-Jun mRNA is regulated by ET-1 in cardiac myocytes (65). Basal levels of c-Jun gene are low in cardiomyocytes; however, c-Jun mRNA and c-Jun protein are rapidly induced by ET-1, and they may be involved in the development of hypertrophic phenotype (65). It is likely that ET-1 induced increase in c-Jun protein is due to c-Jun mRNA (21). These two responses are apparently due to the ERK1/2 cascade which stimulates the expression of c-Jun mRNA by phosphorylation of transcription factors that transactivate at the c-Jun promoter region (21, 66). There are several other consensus sequences including two CRE-like sites and thus c-Jun mRNA could be upregulated by c-Jun (67). Although ET-1 increases c-Jun phosphorylation and inhibition of JNKs reduces the stimulation of c-Jun transcription in myocytes (65). Thus, JNK inhibition does reduce the expression of c-Jun protein as evident by the phosphorylation of c-Jun which may increase the stability by reducing its rate of degradation (65; 67). The regulation of c-Jun abundance and its transactivity by MAPK cascade is still very complex (21).

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4.4. Protein kinases

The protein kinases C (PKCs) are single polypeptide chains and can be structurally divided into an N-terminal-regulatory domain (an auto inhibitory 'pseudoinhibitory' site, and the co-factor/activator-binding sites), a C-terminal catalytic region and a 'hinge' region susceptible to proteolysis (68). There are three PKC subfamilies; the 'classical' or 'conventional' PKCs which require DAG, $Ca2^{+}$ and phosphatidylserine for its activity, the 'novel' PKCs, which are DAG dependent and $Ca2^{+}$independent and the 'atypical' PKCs, which are independent of $Ca2^{+}$ and DAG (68). The cardiac myocytes express nPKCδ and nPKCε, which belong to the DAG-sensitive PKCs (69). It is now known that cPKCα, a conventional PKC, is present in a 10-fold high relative molar abundance as compared to nPKCε (70). Tumor-promoting phorbol esters, like phorbol 12-myristate13 – acetate (PMA) or phorbol 12, 13-dibutyrate, act as pharmacological mimics of DAG which partition into the membrane and are not metabolized (69). They produce a very long lasting and strong association of DAG-sensitive PKCs such as nPKCδ, Cpkc and nPKCε in the cardiac myocytes within the membrane fraction followed by the

loss of DAG-senitive PKCs from the cell over 24 hours (69). When the myocytes are exposed to high concentrations of ET-1 (100nM), nPKCδ and nPKCε translocate stoichiometrically to the membrane fraction within 15-30 seconds (Clerk et al., 1994). As DAG gets phosphorylated nPKCδ returns to the soluble fraction within 1-2 minutes (71). However, nPKCε remains associated with the membrane for 5-15 minutes. The EC_{50} of ET-1 is about 1nM and 10nM for the translocation of nPKCε and nPKCδ respectively. In cardiac myocytes, ET-1 is not able to translocate cPKCδ as compared to the PMA response (71). The most crucial isoforms form of PKCs in cardiac myocytes is perhaps the nPKCε form, because of its propensity to translocate in them (72). In transgenic mice, moderate cardiac myocyte overexpression of constitutively active nPKCε induces compensatory cardiac hypertrophy (72). Although the PKC isoforms, nPKCε and cPKCα have similar specific activities, the translocation of the entire pool of nPKCε would produce same activity effect as would just 10% of the cPKCα pool (21). It has been suggested recently that cPKCα mediates cardiac myocyte hypertrophy in response to ET-1, which can be inhibited by downregulation of cPKCα by antisense methodology (73).

5. APOPTOSIS AND CARDIOMYOCYTES

Apoptosis is a biological phenomenon in which cells respond to damage by triggering programmed cell death. Apoptosis is now considered to be a component in cardiac pathologies including chronic heart failure, cardiac sudden death, ischemia and viral myocarditis (74-76). Over the last 10 years, the role of apoptosis in heart diseases has been explored progressively using both experimental animal models and specimens from the heart disease patients (79-83). Apoptosis is recognized as a fundamental mechanism that is regulated physiologically and genetically. It also plays a central role in the development, immune system function, normal cell turnover and morphogenesis (88, 89).

During apoptosis, a series of profound structural changes occur in the cells (90). One of the earliest events observed microscopically is the condensation of chromatin to form a dense, circumscribed mass in the nucleus (90). Another nuclear- associated event during apoptosis is the degradation of DNA into 180-200-bp of oligonucleosomal fragments (91). As cell volume decreases, cell density increases, nuclear outline and cellular convolution become evident (92). The cytoplasmic changes include cytoskeletal filament aggregation, rearrangement of rough ER to a series of concentric whorls, and clumping of ribosomal particles (93). The shrinkage of cells is also a universal characteristic during apoptosis (94). This occurs due to the movement of the water out of the cell as vesicles bud from the endoplasmic reticulum and Golgi apparatus fuses with the plasma membranes content in and releases its contents into extracellular spaces (94). The active efflux of Na^+ and K^+ ions through Na^+, K^+ ATPase pump and $Ca2^+$-dependent channel is also responsible for shrinkage of cells during apoptosis (95, 96).

The process of apoptosis can be divided into three steps as i) induction, ii) determination, iii) execution (97). Fujiwara and Takemura, in their review article have described these processes in which induction process involves the introduction of an apoptotic stimulus in the cells. A number of chemical and mechanical stimuli such as ligands for Fas and TNF receptors, chemicals, heat and radiation are capable of induction of apoptosis. During determination step, the apoptotic signal is transferred to the nucleus which affects the gene expression of oncogenes such as c-myc, c-fos, bcl-2 and p-53, among other pro-apoptotic genes. Finally, during the execution step, proteolysis of caspases, a family of aspartyl-specific cysteine proteases occurs, leading to DNA fragmentation (98). During cardiac depression induced in sepsis, the cardiac myocytes loose the ability to proliferate; the loss of the cells from apoptosis affects the global cardiac function. Hence, it is essential to maintain the survival of cardiac muscle cells. During normal morphogenesis of the heart, apoptosis plays an important role in its development (106). In embryogenesis, it is required in the morphogenesis of the conducting system, including sinus node, AV node and His bundle (106). In addition, a study was conducted to evaluate the rate of apoptosis in cardiomyocytes and non-cardiomyoctyes (99). It was observed that right ventricle had a greater rate of apoptosis soon after birth as compared to the left ventricles (99).

6. MODULATION OF ENDOTHELIN BIOSYNTHESIS AND SEPSIS

Polymicrobial sepsis in our rodent produced an elevation in ET-1 at 4hr post-sepsis, which declined by 12hr (7). ET-1 precursor bigET-1, showed an increase in plasma and heart ET-1 concentration in vivo (100). In ARVM we observed that biosynthesis of ET-1 produced an elevation of ET-1 more in sham animal as compared to septic animals suggesting that ECE-1 action might be depressed in septic ARVM. On further inhibiting ET-1 biosynthesis by FR901533, an ECE-1 inhibitor, we found depressed biosynthesis of ET-1. This strengthened our initial speculation that ET-1 biosynthesis is depressed in septic myocardium at 24 hr, a hyperdynamic stage of sepsis. However, in our recent study we observed that myocardial ET-1 levels were elevated at 3 and 7 day post-sepsis. This suggests that ET-1 biosynthesis modulation may play a crucial role in organ (myocardium), cellular (ARVM contractility) and vascular dysfunction during sepsis.

6.1. Cardiomyocyte function and signaling mechanism

We have reported that septic ARVMs displayed decreased cell contractility simulated with depressed myocardial performance as observed in isolated heart preparation (7, 8). However, the septic ARVMs at 24-h in culture media displayed increased contractility which could be due to alteration of several biochemical pathways (101). Septic ARVM exhibited upregulated phosphorylation of both p38-MAPK and ERK1/2, suggesting that an increased contractility could be due to MAPK signaling mechanisms or via yet unknown active compensatory mechanism. Further, bigET-1 and FR901533 treatments per se produced

a positive inotropic effect in sham ARVM only but not during sepsis. Further, the ARVM contractility during sepsis was unaltered following pre-treatment with FR901533 and bigET-1 and FR901533 treatments per se. These findings suggest that bigET-1 produced a decompensatory contractile effect during sepsis which was independent of ECE-1 activity (101).

ET-1 is known to induce upregulation of the signaling molecules like ERK1/2 and p38-MAPK in ARVM and neonatal ventricular myocytes (102, 103). We have provided evidence for the involvement of MAPK cascade in ARVM contractility during sepsis (9). ARVM obtained immediately after the isolation from the septic heart displayed decreased cardiac contractility, suggesting a depressed myocardial performance. We observed a pronounced negative inotropic effect of p38- MAPK on contractility in normal (sham) ARVM. SB203580, an inhibitor of p38-MAPK increased ± dL/dT in a dose-dependent manner in sham ARVM. It also increased the peak shortening by ~2-folds in the sham group. However, SB203580 decreased the contractile properties of septic ARVM suggesting that p38-MAPK has an opposite effect on ARVM contractility in sepsis. We found that bigET-1 exerted a positive inotropic effect in sham ARVM and up to 3h in septic ARVM. However, septic ARVM did not show any alterations in peak shortening, ±dL/dT following treatment with bigET-1 at 24h. The unresponsiveness of the positive inotropic effect of bigET-1 on septic ARVM could be due to several reasons. The first reason could be due to the upregulation of p38-MAPK by sepsis (30%) and bigET-1(50%). The data further suggested that bigET-1 during sepsis potentiated the activation of p38-MAPK and produced a ~1.4-fold increase in sham ARVM as compared to vehicle-treated sham ARVM. We also speculated that decreased contractility of septic ARVM in the presence of bigET-1 could be via ET_A receptors. This was further strengthened by observing a downregulation of the ET_A receptors in the septic ARVM. Therefore, we hypothesized that non-responsive effect of chronic bigET-1 during sepsis could be due to downregulation of ET_A receptors and an upregulation of ET_B receptor. Since upregulation of ET_B receptors via mature ET-1 can cause an excessive generation of NO through inducible NOS. We tested the effect of bigET-1 and observed that ET-1 produced an increase in NO. This suggests that ET-1 induced increased NO could be one of the mechanisms responsible for non-responsiveness of bigET-1 on the contractility of septic ARVM (9).

We also found that bigET-1 elevated phosphorylation of p38-MAPK while ECE-1 inhibitor, FR901533, down-regulated phosphorylation of p38-MAPK in the sepsis group (101). Similarly, Kubo et al also demonstrated that ECE-1 inhibitor, phosphoramidon inhibited MAPK activity in vascular smooth muscle cells (104). However, bigET-1 reversed FR901533-induced down-regulation of p38-MAPK phosphorylation in both sham and septic ARVM. Further, a positive inotropic effect on sham ARVM contractility was observed with FR901533 and bigET-1 but not in the sepsis group. Sepsis did not alter ARVM contractility following pre-treatments with FR901533 and bigET-1 and FR-901533 treatment alone (101). This study concluded that chronic exposure of bigET-1 induced p38-MAPK upregulation correlates more to ET-1 induced hypertrophy but not to the ARVM contractility. It is speculated that the observed effects of bigET-1 could be due to both endogenous and exogenous bigET-1 and ET-1 biosynthesis mechanisms play a crucial role in the activation of p38-MAPK in both sham and septic ARVM (101).

Interestingly, an upregulation of signaling molecules such as ERK1/2 is known to increase the survival of neonatal rat ventricular myocytes (105). To elucidate the role of ERK1/2 in bigET-1-induced ARVM contractile response, we incorporated PD98059, an ERK pathway inhibitor in our studies (9, 101). PD98059 produced an increase in the contractile amplitude of ARVM in sham group, suggesting that ERK1/2 also mediates a negative inotropic effect similar to p38-MAPK on sham ARVM (9). Although the PD98059 pretreatment followed by bigET-1 in septic ARVM did not affect the peak shortening and –dL/dT, it did stimulate +dL/dT, suggesting that ERK1/2 pathway is involved along with p38-MAPK in bigET-1 induced ARVM contractility during sepsis (9). Although septic ARVM upregulated the phosphorylation of ERK1/2, our previous studies showed no alterations in the ERK1/2 phosphorylation upon treatment with bigET-1and ECE-1 inhibitor, FR901533 in both sham and septic groups (9).

We demonstrated that bigET-1 produced an increase in caspase-3 activity in both sham and septic animals, along with ARVM hypertrophy and unaltered contractility. Unlike sham, septic ARVMs displayed elevated caspase-3 levels as early as 6-h post-treatment. It was observed that FR901533 pre-treatment with bigET-1 was able to reverse bigET-1 induced caspase-3 activity at 12-h but not at 24-h, suggesting that bigET-1 induced apoptosis is ECE-1 dependent in sham ARVM until 12-h post-treatment. We speculate that in this study, the non-responsiveness of septic ARVM contractile function to bigET-1 could be due to increased caspase-3 activity at 12 and 24-h post-treatment (9).

7. CONCLUSION AND FUTURE PROSPECTIVES

In conclusion, the results from our previous research work have suggested that bigET-1- induced hypertrophy in septic ARVM is through ECE-1 dependent activation of p38-MAPK phosphorylation (figure 1). We have also provided evidence that unresponsiveness of the septic ARVM towards bigET-1, a positive inotrope is due to an ECE-1 dependent increased caspase-3 activity. However, further studies will be required to delineate the pathway for caspase-3 activation and signaling molecules like ERK1/2 and p38-MAPK during early stages of ET-1 activation.

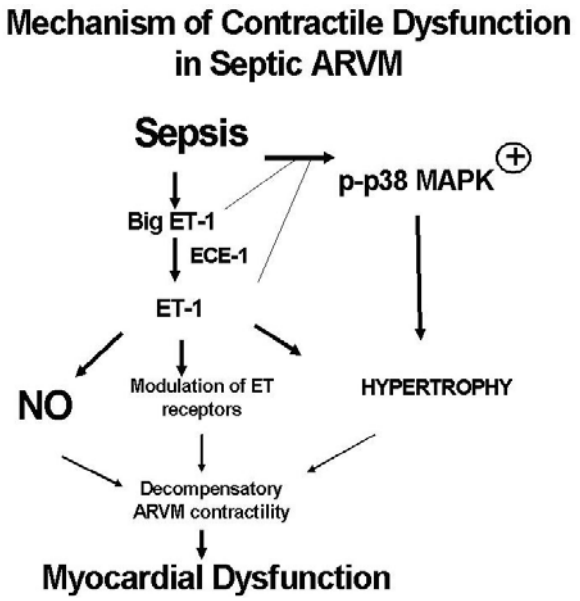

Figure 1. Endothelin (ET) mediated signaling mechanism in adult rat ventricular myocyte (ARVM) following polymicrobial sepsis in the rat. NO, nitric oxide; ECE-1, endothelin converting enzyme-1, p38-MAPK, p38-mitogen activated protein kinase.

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