EFFECT OF EPINEPHRINE ON MYOCARDIAL ISCHEMIA-RELATED GENE EXPRESSIONS IN CULTURED RAT CARDIOMYOCYTES

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Abstract

Epinephrine is a physiologically essential catecholamine for survival and an important pharmacological agent in acute management of cardiac arrest. However, recent studies have demonstrated that prolonged exposure to epinephrine exerts some detrimental effects on the body and cardiac physiology, resulting in adverse clinical outcomes. Our study investigated the effects of *in-vitro* exposure to epinephrine for 48 hours on gene expression in cultured rat cardiomyocytes. We observed an increased expression of three different genes, namely MMP2 (+ 2.092 times), PDE4b (3.881 times) and TNNT2 (2.621 times). Based on the known function of these genes' products, we could deduce that their overexpression can lead to myocardial fibrosis with resultant compromised cardiac contractility and diastolic dysfunction, which may partly be responsible for the observed negative clinical consequences of epinephrine exposure. This preliminary study seems to be inadequate to establish any cause-effect relationship. Further investigations will be needed to elucidate the clinical significance of the observed gene up-regulations.

Introduction

The sympathetic nervous system (SNS) forms the principal neurophysiological setup in the body that regulates and ensures sustainable end-organ perfusion. It does so by constantly adjusting the cardiac output (CO) and vascular tone as responses to the dynamic changes in oxygen demand and other physiological parameters[1,2]. Catecholamines are the neurotransmitters that mediate sympathetic responses, and epinephrine is one of the major catecholamines synthesized within the body[3]. Epinephrine binds directly to multiple adrenoreceptors (AR) including α1, α2, β1 and β2 to exert its sympathetic effects. The α1-adrenoreceptor is coupled to the G_i secondary messenger system that increases intracytoplasmic calcium level in smooth muscle cells and leads to increased vascular tone; whereas the β-adrenoreceptors are linked to the G_s secondary messenger system which increases intracellular cAMP level and leads to improved contractility and higher heart rate

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(HR). Through the above-mentioned physiological changes in HR, contractility and vascular tone, transient elevations in plasma epinephrine level enables the body to cope with the normal physiological stresses of life, such as those experienced during a flight or fight response. However, persistent exposure to elevated levels of epinephrine, both endogenous and exogenous, is known to exert detrimental effects on the body and heart's physiology[4-7]. Increased exposure to endogenous catecholamines occurs following myocardial infarction. This is because myocardial infarction results in a decrease in cardiac output and blood pressure, which leads to sympathetic stimulation and increased release of epinephrine. Initially, the increased epinephrine level helps the heart to compensate for the decrease in cardiac performance and maintain CO and mean arterial blood pressure (MAP). But over the longer term, persistent exposure to epinephrine drives extensive cardiac remodeling that is characterized by cardiac fibroblast proliferation, cardiomyocyte hypertrophy and extracellular matrix (ECM) deposition[4,8]. These pathological changes cause increased cardiac stress and dysfunction resulting in the development and progression of heart failure. In rat models of heart failure, a variety of gene expressions are observed to be increased, that are believed to mediate the profound structural changes associated with the myocardial remodeling[9]. These alterations in gene expressions also include genes that encode for actin and myosin [10], collagen and fibronectin[11,12], atrial natriuretic peptide[13], and cytokines such as interleukin-1β and interleukin-6[14]. Some studies have shown the significant influence of the most prominent catecholamine, norepinephrine, on cardiac remodeling and on the gene alterations underlying the influence[15]. Fewer studies investigated the effect of epinephrine on the ischemic heart. Exposure to exogenous epinephrine has also recently been shown to have adverse long-term clinical outcomes, especially neurologically intact survival and in-hospital mortality[5-7]. Clinical scenarios in which exogenous epinephrine is utilized could include emergent management of cardiac arrest, stabilizing the blood pressure of anesthetized patients intraoperatively, improving MAP and other hemodynamic parameters of patients in shock in the intensive care unit (ICU) setting. Also, exogenous epinephrine is often used as the last and unavoidable resort in critically compromised patients to acutely stabilize their hemodynamics. Although acute and emergent use of epinephrine is associated with early return of spontaneous circulation (ROSC) and potentially improved survival[16-18], its long-term effects and the effects of its inappropriate and excessive clinical use are now being questioned[19,20].

The purpose of this study was to examine the effect of epinephrine on the myocardial ischemia-related gene expressions in cultured rat cardiomyocytes. Also, we aim to provide additional insights into the molecular basis of the adverse clinical outcomes observed in patients with increased exposure to exogenous epinephrine.

Methods

Our cell culture and gene expression study methods were discussed in details in our previous publications[21,22]. Rat cell-line H9C2 cardiomyocytes were for this study. H9C2 cardiomyocytes were used inoculated at the concentration of 0.5M/ml and cultured at 37^{o}C in Dulbecco's Modified Eagle's Medium. The cells were allowed to settle down overnight and then exposed to epinephrine (1uM) for 48 hours. H9C2 cardiomyocytes without epinephrine exposure served as control group. All microarrays were done in triplets. Cardiomyocyte RNA was extracted from the cultured cardiomyocytes and used for whole genome gene expression study. The microarray contains 41,000 + rat genes. cDNA was synthesized from RNA samples and was used to synthesize fluorescent cRNA. The labeled cRNA samples were then hybridized to the Whole Rat Genome Oligo Microarray slides. After hybridization, arrays were washed and scanned. These data were then imported into GeneSpring software as 20 one-color arrays and normalized to the median per chip and the median value per gene across all arrays. Parameter data was added so that the microarrays could be grouped by time and treatment. Guided workflow returned several gene lists. These were statistically analyzed for significant Gene Ontology and pathway hits based on passed P value. Epinephrine-induced gene expressional changes with $P < 0.05$ related to myocardial ischemia were identified and considered statistically significant.

Results

The results of our study are documented in Table 1 and illustrated in Figure 1. Several epinephrine-induced gene expression changes ($P < 0.05$) related to myocardial dysfunction were identified. Epinephrine exposure induced following gene up-regulations: Matrix metallopeptidase-2 (MMP2) was increased 2.092 times; Phosphodiesterase 4b (PDE4b) increased by 3.881 times; Troponin T2 (TNNT2) gene expression increased by 2.621 times.

Table 1
Genes related to myocardial dysfunction with altered expressions in cultured rat cardiomyocytes exposed to epinephrine for 48 hours

Up-regulated gene expressions	Down-regulated gene expressions
MMP2 (2.092 times)	None
PDE4b (3.881 times)	
TNNT2 (2.621 times)	

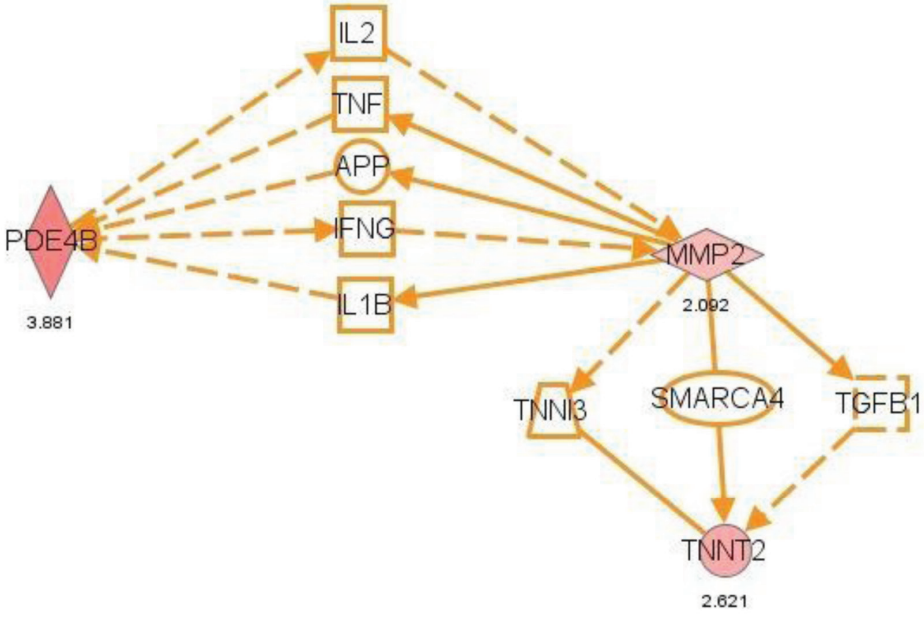
MMP2: Matrix metallopeptidase-2 gene; PDE4b: Phosphodiesterase 4b gene; TNNT2: Troponin T2 gene.

Discussion

Epinephrine, though an essential catecholamine for survival and an important pharmacological agent in acute management of cardiac arrest, has been shown to exert detrimental clinical effects, especially with prolonged exposure[4-7]. This study demonstrated that exposure to epinephrine for 48 hours significantly changed some gene expressions in cultured rat cardiomyocytes. There were increased expressions of three main genes, MMP2, PDE4b and TNNT2. By identifying the genetic changes associated with prolonged epinephrine exposure, our study may help understanding the biological basis of epinephrine induced myocardial ischemia and heart failure.

Matrix metallopeptidase-2 (MMP2) gene encodes for a gelatinase A, type IV collagenase enzyme[24]. This is believed to be a zinc-dependent enzyme which belongs to a family of endopeptidases responsible for cleaving type-IV collagen. Type IV collagen is a major component of the basement membrane. MMP2 can be activated on cellular membrane, extracellularly by proteases and/or intracellularly by S-glutathiolation.

Fig. *1*
Changes in gene expression related to myocradial dysfunction induced after exposure of cardiomyocytes to epinephrine for 48 hours. Red-highlighted indicated increases expression (decreased gene expression is usually green-highlighted, but none was found in this study). The numbers show the times of the gene expression changes



MMP2: Matrix metallopeptidase-2 gene; PDE4b: Phosphodiesterase 4b gene; TNNT2: Troponin T2 gene.

MMP2 contains three fibronectin type II repeats in its catalytic site, which enable it to bind to and cleave denatured type IV collagen. Physiologically, MMP2 gene products are associated with normal tissue remodeling such as embryonic development, reproduction, wound healing, angiogenesis, endometrial menstrual breakdown, and bone formation and reabsorption. Mutations in MMP2 gene can potentially compromise these physiological functions and result in diseases such as multicentric osteolysis, arthropathy and metastases, and abnormal remodeling in cardiac tissues. MMP2 enzyme also modulates cell signaling by degrading ECM. Degradation of ECM results in the release of growth factors that were previously bound to ECM and are subsequently freed to bind to cell receptors and promote cell signaling. In the heart, fibroblasts are the primary cell population and secrete proteins that mainly comprise the ECM[23]. This process of remodeling myocardial matrix is one of the key aspects compromised in chronic cardiac diseases. Hyperactivity of MMP2 and subsequent excessive degradation of ECM will theoretically disrupt the connections between myocardial cells and blood vessels in the myocardium, therefore compromising myocardial structural integrity[24]. On the other hand, decreased MMP2 activity will likely result in excessive ECM depositions leading to myocardial fibrosis with resultant diastolic dysfunction of the heart[24]. Myocardial ischemia is usually associated with increased release of MMP2, and the MMP2 level generally correlates negatively with the heart's recovery of mechanical function[25]. Some evidence suggests MMP2 contributes towards cardiac contractile dysfunction by proteolytic degradation of the thin filament protein, troponin I[26] and the myofilament protein, titin[27]. Inhibition of MMP2 could help prevent progression of cardiac ischemia to heart failure[28]. Our study showed that MMP2 gene expression level was up-regulated, which provided some more supportive evidence to the above investigation results at the gene expression level.

Phosphodiesterase 4b (PDE4b) gene encodes for a Type IV phosphodiesterase (PDE) enzyme. This enzyme belongs to a family of phosphodiesterase, which are usually found in the heart tissues and they are responsible for hydrolyzing cyclic adenosine monophosphate (cAMP) specifically within the myocardial tissue. Cyclic AMP is synthesized by adenylyl cyclase and degraded by phosphodiesterase (PDE) via hydrolysis [29]. The cAMP secondary messenger system is pivotal for mediating autonomic regulation of the heart, especially sympathetically driven increases in myocardial contractility[29-31]. cAMP works by activating enzyme protein kinase A, which in turn phosphorylates several proteins involved in cardiac excitation-contraction cycle, as L-type calcium channels, troponin I, myosin binding protein C, ryanodine receptors, and phospholamban[29]. L-type calcium channel phosphorylation and ryanodine receptors phosphorylation result in increased uptake of extracellular calcium and increased calcium release of sarcoplasmic reticulum respectively. Both could result in improved myocardial contraction by enhancing available cytoplasmic calcium. Phosphorylation of phospholamban can increase the uptake of calcium by sarcoplasmic reticulum, leading to improved myocardial relaxation. Phosphorylation of Troponin I may decrease its affinity for calcium and results in faster dissociation of calcium with faster muscular relaxation[32]. Phosphorylation of myosin-binding protein C can also enhance cross-bridge cycling[33]. Through these mechanisms, cAMP supports both the inotropic and lusitropic effects of exogenous epinephrine and intrinsic sympathetic stimulation. Other potential aspects of cardiomyocyte physiology such as gene transcription are also regulated by cAMP[34]. Thus, overexpression of PDE4b, as discovered by our study, can potentially limit cAMP signaling and blunt the inotropic response to sympathomimetic stimulations, thus leading to myocardial dysfunction and heart failure[35-37].

Troponin T2 (TNNT2) gene encodes for myocardial troponin T protein, the tropomyosin binding subunit of the troponin complex[38]. The troponin complex plays an important role in mediating muscle contraction via actin-myosin interactions[39]. This complex is located on the thin actin filament of striated muscles and is composed of three subunits, Troponin I, T and C[40]. Troponin T usually prevents actin-myosin interactions by binding to tropomyosin, which conceals the myosin binding site of actin. Troponin C, on the other hand, enables actin-myosin interaction by exposing the myosin-binding site of actin[41]. TNNT2 gene mutations are associated with 15%

of all familial cases of hypertrophic cardiomyopathy, while myosin heavy chain (MYH7) gene mutations are a more common cause of familial hypertrophic cardiomyopathy. TNNT2 gene mutations are believed to cause more severe phenotypes with complete penetrance of familial hypertrophic cardiomyopathy, and they are linked to a higher incidence of sudden cardiac death even with only minimal hypertrophy[42,43]. TNNT2 gene mutations are also associated with dilated cardiomyopathy, which results in heart failure. The exact mechanism by which TNNT2 gene mutation leads to cardiomyopathy is not fully elucidated yet, but it seems to be caused by compromised sarcomere assembly and myocyte disarray during embryonic development[42,44].

Based on the gene expressional alterations we observed in this study, prolonged epinephrine exposure appears to affect the following areas: increased MMP2 gene expression may increase ECM deposition, leading to myocardial fibrosis and thus causing diastolic cardiac dysfunction, and likely abnormal myocardial remodeling after myocardial ischemia. Enhanced gene expression of PDE4b may negatively affect the myocardial contractility leading to heart failure. Increased gene expression of TNNT2 seems to be associated with increased incidence of cardiomyopathy. All these may potentially be contributing to the observed negative effects of epinephrine on the long-term clinical outcomes. Obviously, this is a preliminary study that does not provide a definitive cause-effect relationship. Further experimental and clinical investigations are surely needed to elucidate the clinical implications of these altered gene expressions and their potential clinical impact on our practice.

Conclusion

We found that there are three genes that showed increased expressions: MMP2, PDE4b and TNNT2. The overexpression of these three genes are potentially associated with increased ECM deposition leading to myocardial fibrosis and diastolic dysfunction, compromised myocardial contractility, and potentially increased incidence of cardiomyopathy. Further studies are needed to elucidate the precise consequences of the observed gene upregulations and their clinical implications.

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