Adrenergic regulation of myocardial apoptosis

Krishna Singh, Catherine Communal, Douglas B. Sawyer, Wilson S. Colucci*

Myocardial Biology Unit and Cardiovascular Division, Boston University Medical Center, Boston Veterans Affairs Medical Center and Boston University School of Medicine, 88 East Newton Street, Boston, MA 02118, USA

Received 12 August 1999; accepted 11 October 1999

Abstract

Increased sympathetic nerve activity to the myocardium is a central feature in patients with heart failure. Norepinephrine, the primary transmitter of the sympathetic nervous system, signals via binding to α- and β-adrenergic receptors (AR) that are coupled to G-proteins. Pharmacologic studies of cardiac myocytes in vitro demonstrate that β-AR can stimulate apoptosis. Likewise, in transgenic mice overexpression of β₁-AR or Gαs is associated with myocyte apoptosis and the development of dilated cardiomyopathy. Whereas β₁-AR stimulate apoptosis in vitro and in vivo, β₂-AR may either stimulate or inhibit apoptosis and myocardial failure depending on the level of expression. Receptors coupling to Gi and Gq may also be able to mediate or modulate apoptosis and the development of myocardial failure, suggesting the potential for interactions between the β-AR system and numerous remodeling stimuli that act through Gi or Gq signaling pathways. It appears likely that the mitogen-activated protein kinase superfamily plays a key role in mediating the actions of adrenergic pathways on myocyte apoptosis. These observations suggest that the adrenergic nervous system plays an important role in the regulation of myocyte apoptosis, and may thus contribute to the development of myocardial failure. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Adrenergic (ant)agonists; Apoptosis; Autonomic nervous system; G-proteins; Heart failure; Receptors; Signal transduction

1. Introduction

The finding of apoptosis in hearts from patients with end-stage heart failure [1,2] has raised the possibility that apoptosis contributes to the pathophysiology of myocardial failure. Apoptosis of cardiac myocytes during fetal and early postnatal development may play a central role in determining the number of myocytes in the adult heart [3]. However, the apparently limited capacity for regeneration of myocytes in the adult heart suggests that continued myocyte loss due to apoptosis could contribute to progressive myocardial failure.

Increased sympathetic nerve activity in the myocardium is a central feature in patients with heart failure [4,5]. Norepinephrine (NE), the primary transmitter of the sympathetic nervous system, binds to G-protein-coupled adrenergic receptors (AR), of which at least nine subtypes have been identified [6,7]. Here we will focus on the potential role of AR and their associated G-proteins in mediating apoptosis in cardiac myocytes.

2. NE cardiotoxicity

As early as 1959 Rona et al. [8] and others [9–11] observed that chronic exposure of rats to adrenergic agonists caused myocyte necrosis in the absence of obstructive coronary artery narrowing. In 1992, Mann et al. [12] extended these observations to adult feline cardiac myocyte in primary culture. They found that when quiescent rod shaped cardiac myocytes in culture were exposed to NE a large proportion contracted spontaneously, developed hypercontracture and subsequently died over the next 2 to 3 days. This cytotoxic effect of NE, which was...
associated with the release of creatine kinase, was antagonized by the \(\beta\)-AR antagonist propranolol (Fig. 1), but not the \(\alpha\)-AR antagonist phenolamine, and was mimicked by the \(\beta\)-AR selective agonist isoproterenol. They further demonstrated that cAMP and calcium influx via verapamil-sensitive channels were necessary for the cytotoxic effect of NE.

3. \(\beta\)-AR stimulation increases myocyte apoptosis

3.1. Adult rat cardiac myocytes in vitro

Using adult rat cardiac myocytes in vitro, we found that the cardiotoxic effect of NE was associated with apoptosis [13]. Exposure to NE (10 \(\mu\)M) for 24 h increased genomic DNA fragmentation as analyzed by agarose gel and the percentage of cells that were stained positive by terminal deoxyxynucleotidyl transferase-mediated nick end-labeling (TUNEL) staining. These changes were associated with an increase in the percentage of apoptotic cells with hypodiploid DNA content as measured by flow cytometry. The apoptotic effect of NE was abolished by the \(\beta\)-AR antagonist propranolol, but not the \(\alpha\)-AR antagonist prazosin (Fig. 2). The apoptotic effect of NE was mimicked by the \(\beta\)-AR agonist isoproterenol and by forskolin, a direct activator of adenyl cyclase. The protein kinase A (PKA) inhibitor H89 likewise blocked the apoptotic effects of NE, as did the L-type calcium channel blocker diltiazem. These studies indicate that NE, acting via the \(\beta\)-AR pathway, stimulates apoptosis in adult cardiac myocytes and that the effect is mediated by the activation of protein kinase A and requires calcium entry via the voltage-dependent calcium channel.

3.2. Isoproterenol infusion in vivo

In 1998, Shizukuda et al. [14] found a similar effect of \(\beta\)-AR stimulation in vivo. They observed that continuous infusion of isoproterenol for periods of 12 h to 7 days increased the frequency of TUNEL-positive myocytes in the heart at all time points. Most of the TUNEL-positive cardiac myocytes were located in areas of myocardial damage. Pacing to the same heart rate as stimulated by isoproterenol did not increase the number of apoptotic myocytes, suggesting that apoptosis was not due to tachycardia.

4. Differential effects of \(\beta\)-AR subtypes

4.1. \(\beta_1\)- vs. \(\beta_2\)-AR

Recently, using adult rat cardiac myocytes in vitro we found that the \(\beta_1\)-AR selective antagonist CGP 20712A completely prevented NE-stimulated apoptosis, whereas the \(\beta_2\)-AR selective antagonist ICI 118,551 potentiated the amount of NE-stimulated apoptosis [15]. Interestingly, in this system pretreatment with pertussis toxin (PTX) to inactivate Gi increased the magnitude of \(\beta\)-AR stimulated...
apoptosis, and thus mimicked the effect of a β2-AR selective antagonist. Conversely, we found that pretreatment with carbachol, which stimulates Gi via activation of M2 muscarinic receptors, inhibited β-AR-mediated apoptosis [15]. Thus, at least in this experimental model, NE stimulates apoptosis via β1-AR and inhibits apoptosis via β2-AR. The later action appears to involve a PTX-sensitive G-protein, most likely Gi.

The opposing effects of β1- vs. β2-AR on myocyte apoptosis may be due to the differential signaling of these receptors subtypes. Although both β1- and β2-AR couple to Gs and exert effects via adenyl cyclase and cAMP, a component of the effects of β2-AR-stimulation may oppose the actions of Gs and appears to be independent of Gs and cAMP [16–18]. These observations may relate to the recent demonstration that β2-AR, but not β1-AR, also couple to a PTX-sensitive G-protein, presumably Gi [19]. For example, in cardiac myocytes pretreatment with PTX potentiates the response to β2-AR, not β1-AR, stimulation [19,20]. It is possible that coupling of β2-AR to Gi could oppose actions mediated by the coupling of β1- and β2-AR to Gs. Interestingly, the coupling of β1-AR to Gi may be regulated by a Gs-mediated, protein kinase A-dependent phosphorylation of the β2-AR which favors coupling to Gi [21]. In the adult rat cardiac myocyte system that we studied, the net effect of NE is to increase the frequency of apoptosis, presumably reflecting the predominance of β1-AR in these cells [13,15].

4.2. β1-AR

Relatively little is known about the functional role of β1-AR in the myocardium. Since they appear to act in part by stimulation of nitric oxide [22], β1-AR have the potential to modify calcium influx, reactive oxygen species and a variety of biochemical pathways that might influence the rate of apoptosis.

5. Neonatal rat cardiac myocytes

It is not clear whether β-AR stimulation affects the frequency of apoptosis in neonatal rat ventricular myocytes. In one report, NE alone had no effect on the frequency of apoptosis, but inhibited apoptosis stimulated by atrial natriuretic peptide (ANP) [23]. The antiapoptotic effects of NE on ANP-stimulated apoptosis were blocked by propranolol but not by the α1-selective antagonist terazosin. These antiapoptotic effects of NE were mimicked by isoproterenol and dibutryl-cAMP.

However, Iwai-kanai et al. have recently reported that in neonatal rat cardiac myocytes β-adrenergic stimulation increases apoptosis [24]. The apoptotic action of β-adrenergic stimulation was opposed by concurrent treatment with an α-adrenergic agonist. The reason for the discrepant findings in neonatal cardiac myocytes remains to be determined, but may relate to differences in the cell culture conditions.

6. Adrenergic receptor overexpression in mouse myocardium

Work with transgenic mice that overexpress AR receptors and G-proteins in the myocardium has provided insights into the mechanism by which adrenergic activation can affect myocyte phenotype.

6.1. β1-AR overexpressing mice

At least two groups have made mice that overexpress the β1-AR in the myocardium under the control of the α-myosin heavy chain (MHC) promoter [25,26]. In both cases the transgenic mice are born and develop normally. They exhibit increased cardiac contractility at a young age associated with myocyte hypertrophy [26]. However, by about 9 months of age they develop left ventricular dilation with decreased contractile function [25]. Recently, Port et al. (unpublished data) observed that mice overexpressing β1-AR in the myocardium have an increased frequency of apoptotic myocytes as measured by in situ TUNEL-staining.

6.2. β2-AR overexpressing mice

Mice overexpressing β2-AR in the heart also exhibit increased contractile function when young [19]. In contrast to β1-AR overexpressing mice they do not appear to develop myocardial dysfunction with age, although the duration of these observations (approximately 4 months) is not sufficient to conclude that an adverse effect will not occur with time.

However, in mice that overexpress Goq in the myocardium, the concurrent overexpression of β2-AR exerts biphasic, level-dependent effects on phenotype. As discussed below, myocardial overexpression of wild-type Goq (Goq-25) results in cardiac hypertrophy, fetal gene expression and progressive ventricular failure [27–30]. With the concurrent overexpression of a low level of β2-AR there is rescue of the phenotype with improved ventricular function and reduced fetal gene expression [31]. In contrast, with the concurrent overexpression of a high level of β2-AR there is marked acceleration of the phenotype with rapid progression to heart failure and death at 5 weeks of age. Mice expressing an intermediate level of β2-AR had increased adenyl cyclase activity but no change in hypertrophy, fetal gene expression or ventricular function. These data are consistent with the thesis that β2-AR exert counterbalancing effects on phenotype. At low levels of receptor expression, there is improved ventricular function and possibly coupling to a protective signaling mechanism, whereas at high levels of receptor
expression excessive activation of adenylyl cyclase tips the balance in favor of the failure phenotype.

6.3. \( \alpha_1 \)-AR overexpressing mice

Mice that overexpress a constitutively-active \( \alpha_{1B} \)-AR in the myocardium develop myocardial hypertrophy with myocyte widening and increased expression of ANP at 10 weeks of age [32]. In contrast, expression of a wild-type \( \alpha_{1B} \)-AR does not cause myocardial hypertrophy despite increased expression of ANP [33,34]. In these mice the contractile response to \( \beta \)-AR stimulation was blunted, as was basal and stimulated adenylyl cyclase activity in isolated membranes. Of note, the attenuated \( \beta \)-AR signaling was reversed by treatment of mice with PTX, suggesting that \( \alpha_{1B} \)-AR overexpression had led to increased activity of Gi.

7. G-protein overexpression in mouse myocardium

The intracellular signaling by which sympathetic nerve activity regulates myocardial function involves coupling of receptors to GTP-binding proteins (G-proteins) [6]. Both \( \beta_1 \) and \( \beta_2 \)-AR signal through coupling of the stimulatory G-protein (Gs) to adenylyl cyclase, leading to production of cAMP. It has recently been shown that \( \beta_2 \)-AR can also couple to other signaling pathways independent of cAMP or Gs, and in particular, to a PTX-sensitive pathway thought to be mediated by Gi [20,21,35,36]. The \( \alpha_1 \)-AR couples primarily through Gq and Goi [6].

7.1. Gas overexpressing mice

Vatner and colleagues developed transgenic mice with an approximately threefold overexpression of Gs alpha in the myocardium associated with a modest (88%) increase in Gs activity [37]. Although there was no increase in basal or stimulated adenylyl cyclase activity, there was a decrease in the lag time to activation by GppNHp. Furthermore, the ionotropic and chronotropic responses to exogenous sympathetic stimulation were increased in the transgenic animals [37]. By 16 months of age there was myocardial degeneration and atrophy, replacement fibrosis and hypertrophy of myocytes with increased cross-sectional area [37]. These histological changes were associated with chamber dilation, reduced ejection fraction and increased mortality [38].

Histochemistry and electron microscopy revealed the presence of myocytes with abnormal nuclei characterized by chromatin condensation, vacuole formation and irregularity of the nuclear membrane [39]. TUNEL-staining revealed an increase in the number of TUNEL-positive myocyte nuclei in the 15–18 month old transgenic mice (Fig. 3) whereas mice 4–7 months of age exhibited only rare TUNEL-positive cells in a frequency not different from wild-type animals. Similarly, cardiac myocytes isolated from newborn Gs alpha transgenic mice showed an increased number of TUNEL-positive nuclei and internucleosomal DNA when treated with the \( \beta \)-agonist isoproterenol [39]. These studies led to the suggestion that excessive activation of Gs alpha could contribute to the development of cardiomyopathy due to apoptosis.

7.2. Gq overexpressing mice

NE induces hypertrophy in cardiac myocytes in vitro via \( \alpha_1 \)-AR which couple to Gq, thereby activating phospholipase C, inositol 1,4,5-triphosphate and protein kinase C [6]. Transfection of neonatal cardiac myocytes with constitutively-active Gq increased ANP expression, whereas injection of an neutralizing Gq antibody blocked \( \alpha_1 \)-AR-stimulated hypertrophy and ANP expression [40]. Gq couples to several other agonists including angiotensin II and endothelin [41].

Dorn and colleagues developed mice that overexpress wild-type Gq in the myocardium under the control of the alpha-myosin heavy chain promoter [27,29,30]. In heterozygous animals with a four- to fivefold increased level of Gq there was myocardial hypertrophy [30] associated with a normal life span [29], although ventricular myocytes isolated from these mice exhibited depressed contractility [29]. Interestingly female mice overexpressing Gq developed a fulminant form of peripartal heart failure associated with myocyte apoptosis and fibrosis [27]. However, when heterozygotes were crossed, yielding mice with an eightfold increase in myocardial Gq levels, the animals died from heart failure at an average age of 11 weeks [27]. Of note, these animals had a marked increase in the
frequency of apoptotic myocytes, leading to the suggestion that apoptosis had contributed to myocardial failure.

It was further found that if mice overexpressing Gq were exposed to pressure overload (aortic constriction) they developed a different phenotype than wild-type animals. In nontransgenic mice aortic constriction resulted in concentric hypertrophy with maintained cardiac performance. In marked contrast, in Gq overexpressing mice pressure overload resulted in eccentric hypertrophy associated with depressed contractile function and hemodynamic decompensation leading to heart failure [29].

Mende et al. [28] developed transgenic mice expressing an epitope-tagged, constitutively-active mutant of Gq under the control of the α-myosin heavy chain promoter. They found that the expression of the epitope-tagged transgene decreased over time and was not detectable by 10 weeks. Nevertheless, the animals developed cardiac hypertrophy, progressive ventricular dilation and died from heart failure at 8–30 weeks of age, leading to the suggestion that even transient overexpression of Gq could initiate a pathological sequence leading to cardiac decompensation.

7.3. Mice overexpressing Gq inhibitor

Transgenic mice with cardiac specific expression of a Gq inhibitor transgene (carboxy-terminus of Gq, residues 305–359) exhibited less hypertrophy in response to pressure overload (transverse aortic constriction) than did wild-type mice, further supporting a role for Gq in the regulation of myocardial hypertrophy [42].

8. Role of mitogen-activated protein kinases (MAPKs)

The MAPK superfamily has been shown to play an important role in the regulation of cardiac myocyte hypertrophy and apoptosis [43]. The MAPK superfamily includes extracellularly responsive kinases (ERKs) and the two stress-responsive MAPK (SR-MAPKs) subfamilies, the c-Jun N-terminal kinases (JNKs) and p38-kinases [43]. JNKs are 46 or 54 kDa proteins. Six isoforms (α1, α2, β1, β2, γ and δ) of p38-kinases have been cloned. Human heart expresses primarily the α and β isoforms of p38- kinase [44].

G-protein coupled receptors have been shown to activate ERKs and the SR-MAPKs [21,41,43]. Of particular interest is the demonstration by Daaka et al. [21] that PKA-mediated phosphorylation of β2-AR induces coupling to Gi, which initiates signaling events through βγ-subunits to activate ERK pathways. βγ-Subunits are also known to activate JNKs via Ras in a Rac1-dependent manner [45]. Likewise, expression of a constitutively-active Giα-2 subunit activates SR-MAPKs including p38 kinases and JNKs [46]. These observations suggest that β2-AR are capable of activating the MAPKs module via coupling with Gi.

The SR-MAPKs appear to play a key role in regulation of hypertrophy and apoptosis in cardiac myocytes. In neonatal rat ventricular myocytes the expression of constitutively-active MAPK kinase (MKK7), an upstream activator of JNKs, caused sarcomeric organization, increased cell size and enhanced the expression of ANP [47]. The expression of a constitutively-active p38β isoform caused a hypertrophic response, whereas expression of a constitutively-active p38α isoform caused apoptosis [48]. It appears that activation of the ERKs cascade is not sufficient for triggering a hypertrophic response for G-protein coupled receptors [49]. The relative roles of ERKs and SR-MAPKs in stimulating or opposing apoptosis remains to be determined.

9. Clinical implications

β-AR antagonists have been shown to improve left ventricular remodeling, ameliorate symptoms and improve survival in patients with systolic myocardial failure. Basic research in cardiac myocytes in vitro and transgenic mice demonstrates that β-AR and Gαs can stimulate myocyte apoptosis associated with development of the failure phenotype. Thus, the data so far support the thesis that increased sympathetic tone contributes to progressive myocardial failure in patients via stimulation of the β-AR/ Gαs pathway.

Certain caveats should be kept in mind. First, these studies do not prove that the deleterious effect of β-AR stimulation is due to apoptosis, or conversely, that the beneficial effect of β-AR blockade is due to reduced apoptosis. Second, the specificity of the indices of apoptosis commonly used in most studies is not certain. For example, it has been pointed out that a positive TUNEL reaction can reflect DNA repair [50] or oncocyte necrosis [51].

Likewise, many issues remain to be clarified. While β1-AR seem only to stimulate apoptosis, β2-AR appear capable of either stimulating or inhibiting apoptosis, and may accelerate or retard the development of myocardial failure. It further appears that receptors coupling to Gi and Gq have the potential to mediate or modulate apoptosis and the development of myocardial failure, suggesting the potential for interactions between the β-AR system and numerous remodeling stimuli that act through Gi or Gq signaling. Finally, it appears likely that the MAPK superfamily plays a key role in mediating the actions of adrenergic pathways on myocyte apoptosis and phenotype.

Taken together, these exciting new insights suggest that greater understanding of the mechanism by which the adrenergic nervous system regulates myocyte apoptosis will lead to even further improvement in the treatment of heart failure.
Acknowledgements

This work is supported by NIH grants HL57947 (KS); HL42539, HL52320 and HL61369 (WSC); HL03878 (DBS); and by a Fellowship (CC) and Grant-in-Aid (KS, DBS) from the American Heart Association, New England Affiliate, and a Merit Review Grant from the Department of Veterans Affairs (KS).

References

[36] Iwase, M.; Bishop, R.H.; Uechi, M. et al. Adverse effects of chronic...


