High Levels of Circulating Epinephrine Trigger Apical Cardiodepression in a β_2-Adrenergic Receptor/G_i–Dependent Manner

A New Model of Takotsubo Cardiomyopathy

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Background—Takotsubo cardiomyopathy is an acute heart failure syndrome characterized by myocardial hypocontractility from the mid left ventricle to the apex. It is precipitated by extreme stress and can be triggered by intravenous catecholamine administration, particularly epinephrine. Despite its grave presentation, Takotsubo cardiomyopathy is rapidly reversible, with generally good prognosis. We hypothesized that this represents switching of epinephrine signaling through the pleiotropic β_2-adrenergic receptor (β_2AR) from canonical stimulatory G-protein–activated cardiostimulant to inhibitory G-protein–activated cardiodepressant pathways.

Methods and Results—We describe an in vivo rat model in which a high intravenous epinephrine, but not norepinephrine, bolus produces the characteristic reversible apical depression of myocardial contraction coupled with basal hypercontractility. The effect is prevented via G_i inactivation by pertussis toxin pretreatment. β_2AR number and functional responses were greater in isolated apical cardiomyocytes than in basal cardiomyocytes, which confirmed the higher apical sensitivity and response to circulating epinephrine. In vitro studies demonstrated high-dose epinephrine can induce direct cardiomyocyte cardiodepression and cardioprotection in a β_2AR-Gi–dependent manner. Preventing epinephrine-G_i effects increased mortality in the Takotsubo model, whereas β-blockers that activate β_2AR-G_i exacerbated the epinephrine-dependent negative inotropic effects without further deaths. In contrast, levosimendan rescued the acute cardiac dysfunction without increased mortality.

Conclusions—We suggest that biased agonism of epinephrine for β_2AR-G_s at low concentrations and for G_i at high concentrations underpins the acute apical cardiodepression observed in Takotsubo cardiomyopathy, with an apical-basal gradient in β_2ARs explaining the differential regional responses. We suggest this epinephrine-specific β_2AR-G_i signaling may have evolved as a cardioprotective strategy to limit catecholamine-induced myocardial toxicity during acute stress. **(*Circulation*. 2012;126:697-706.)**

Key Words: acute heart failure ■ catecholamines ■ receptors, adrenergic, beta ■ Takotsubo syndrome

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There has been a rapid increase in the recognition of a syndrome of acute and severe but reversible heart failure called Takotsubo or stress cardiomyopathy,[1–3] also known as broken heart syndrome, which usually follows within hours of an identifiable emotional, psychological, or physical stress. Takotsubo cardiomyopathy mimics symptoms of acute myocardial infarction but is distinguished by the lack of coronary occlusion and by characteristic regional wall-motion abnormalities, classically a virtual apical ballooning appearance caused by a hypercontractile base of the heart relative to hypokinetic or akinetic apical and mid left ventricular myocardium, the latter extending beyond a single coronary artery territory.[1,2] Initial

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recognition in earthquake survivors in Japan, plus the characteristic ventricular shape, led to the Takotsubo (meaning *octopus pot*) label.[3,4] Approximately 1% to 2% of all presentations with suspected acute coronary syndrome cases are ultimately diagnosed as Takotsubo cardiomyopathy.[3]

Clinical Perspective on p 706

The pathophysiological mechanisms for this increasingly recognized syndrome are not known. Evidence points to epinephrine as the precipitating factor. Physical or psychological stress is a frequent precipitant, and serum catecholamine levels in Takotsubo patients 1 to 2 days after presentation are higher than those in patients with myocardial infarction with pulmonary edema; epinephrine returns to myocardial infarction levels only after 7 to 9 days.[1] Catecholamine storms, more associated with epinephrine-secreting pheochromocytomas than norepinephrine- and dopamine-secreting pheochromocytomas,[5] can also precipitate Takotsubo cardiomyopathy.[6] In particular, the reproduction of the signs of Takotsubo by accidental administration of epinephrine (including single intramuscular 1-mg doses from an epinephrine autoinjector, or EpiPen) is most indicative of its central role.[7] Although there is significant mortality in the early period (1%–1.5%), there is also a characteristic rapid (days to weeks) recovery of patients who survive the acute period of profound depression in left ventricular contractile function,[1] with excellent prognosis and absent or minimal residual cardiac impairment. This striking difference from the normal prognosis of heart failure led us to propose previously that the cardiodepression has elements derived from a beneficial physiological protective adaptation.[8] Thus, the syndrome has interest for the cardiologist over and above the design of optimal treatment for the individual Takotsubo patient.

We have previously proposed a mechanism based on 2 overarching principles for which there is prior evidence. First, the mammalian left ventricle contains apical-basal gradients of β-adrenergic receptors (βARs) and sympathetic innervation, with the apex characterized by highest βAR and lowest sympathetic nerve density.[8] Rat, feline, rabbit, and dog left ventricles show increased apical responses to global high-dose isoproterenol challenges,[9–12] with increased apical versus basal βAR levels measured directly in the dog ventricle.[10] This pattern results in increased apical responsiveness to circulating catecholamines, predominantly epinephrine from the adrenal glands, as a compensatory mechanism for the sparse apical sympathetic innervation, to ensure optimal ventricular ejection during times of stress. Conversely, sympathetic innervation is highest in the basal myocardium and lowest in the apex and therefore cannot explain the localized apical dysfunction. This is also true of human left ventricle,[13] although the presence of a ventricular cardiomyocyte βAR gradient in the human heart remains to be determined.

Second, epinephrine, at high levels, can act as a negative inotrope via ligand-mediated trafficking of the β_2AR from stimulatory G protein to inhibitory G protein subcellular signaling pathways. The β_2AR is widely reported as being pleiotropic, having the potential to couple through G_s-adenylate cyclase-cAMP (like the β_1AR) but also though $G_{i\alpha}$, $G_{\beta\gamma}$, and non–G-protein pathways.[14,15] β_2AR-G_i–mediated depression of contraction was initially demonstrated with transgenic mice that overexpressed β_2AR (TGβ_2).[16,17] At high epinephrine concentrations, the β_2AR switches its coupling from G_s protein to an inhibitory G_i protein,[16] a process described as ligand- or stimulus-directed trafficking, or biased agonism. This switch would be favored in conditions of high catecholamine stress because it depends on β_2AR phosphorylation by both protein kinase A (PKA)[18] and G-protein receptor–coupled kinases (GRKs).[19] This is particularly relevant given the increased frequency of the L41Q GRK5 polymorphism, known to increase cardiac GRK5 activity and βAR phosphorylation, in a recent study that genotyped patients with Takotsubo cardiomyopathy.[20] The negative inotropic effect through G_i[21,22] has contributions both from inhibition of G_s-cAMP production and through other pathways such as p38 mitogen-activated protein kinase (MAPK) alteration of myofilament sensitivity.[23] No such role for β_1ARs in this G_s-G_i trafficking switch has been documented, and the phenomenon is epinephrine specific. Norepinephrine has 20-fold lower affinity for the β_2AR compared to the β_1AR, with much weaker β_2AR stimulus trafficking to the G_i pathway.[16] Although this negative inotropy is detrimental from a mechanical perspective, the G_s-to-G_i switch is potentially both antiapoptotic and antiarrhythmic[24,25] and may represent a cardioprotective mechanism against β_1AR-catecholamine cardiotoxicity. Both the p38 MAPK and phosphatidylinositol 3-kinase/Akt pathways have been implicated in β_2AR-G_i–mediated antiapoptotic effects in adult cardiac myocytes,[26,27] and evidence for increased phosphatidylinositol 3-kinase and Akt activation has been reported in myocardial biopsy samples from patients with Takotsubo cardiomyopathy during the acute phase.[28] Interestingly, direct negative inotropic effects of some β-blockers in human ventricular cardiomyocytes have been shown to depend on β_2AR-G_i signaling,[29] an observation that may have implications for their use in Takotsubo cardiomyopathy.

In the present study, we have developed an epinephrine-induced in vivo model of Takotsubo cardiomyopathy that reproduces both the apically located negative inotropism and the reversible nature of this cardiodepression. We have used this to explore the role of β_2AR apical-basal gradients, the involvement of G_i signaling, and the cardioprotective nature of this condition. It has been supplemented by an in vitro model of acute epinephrine exposure to explore underlying cellular mechanisms. Potential pharmacological agents have been assessed in terms of treatment of the established Takotsubo cardiomyopathy, with the intention to mitigate the cardiodepression without disrupting any of the cardioprotective elements of the syndrome.

Methods

All studies complied with the United Kingdom Home Office regulation governing the care and use of laboratory animals and with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996).

In Vivo Takotsubo Cardiomyopathy Model

Adult male Sprague-Dawley rats (weight 250–350 g) were anesthetized and injected with epinephrine 4.28×10^{-8} mol/100 g or norepinephrine 1.43×10^{-7} mol/100 g via the right jugular vein as a

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bolus injection. Regional left ventricular responses were recorded by 2-dimensional echocardiography (VisualSonics Vevo 770) in the parasternal long axis. Baseline scans were performed before catecholamine administration. For preventative studies, a subgroup of animals were pretreated with the G_i protein inhibitor pertussis toxin (PTX; 25 μg/kg), the p38 MAPK antagonist SB203580 (0.1–10 μg/kg), or the β_2AR-selective antagonist ICI-118,551 (1 μg/kg) followed by intravenous epinephrine bolus. A separate cohort of case animals had continuous aortic blood pressure recording during the protocol with a 1.9F pressure-volume catheter (Scisense Inc). For rescue strategies, a subgroup of animals were treated with intravenous propranolol (1.43×10^{-11} mol/100 g), carvedilol (1.43×10^{-11} mol/100 g), or levosimendan infusion ($4.7\ \mu g\cdot kg^{-1}\cdot min^{-1}$) 15 minutes after epinephrine injection.

Rat Cardiomyocyte Isolation and β_2AR-Overexpression Studies

Myocytes were isolated from adult male Sprague-Dawley rats (Harlan, Bicester, United Kingdom; weight, 250–350 g) by the standard enzymatic technique described previously.[30] Isolated rat cardiomyocytes were plated in culture medium at a field density of 10 000 cells/well and infected with either adenovirus containing β_2AR and green fluorescent protein (Ad.β_2AR.GFP); β_2AR with mutations at the PKA phosphorylation sites 261, 262, 345, and 346 S/A (β_2AR-PKA-KO; Ad.β_2AR-PKA-KO); or adenovirus containing GFP (Ad.GFP; control) at a multiplicity of infection of 500 for 48 hours. For PTX treatment, Ad.β_2AR.GFP-infected rat ventricular myocytes were cultured in the presence or absence of PTX (1.5 μg/mL) for 48 hours. Survival in culture was shown as a percentage of rod-shaped myocytes at the time of plating; >100 cells per well were counted, with triplicates for each condition. β_2AR-specific contractile responses were measured on separately isolated apical and basal ventricular cardiomyocytes with isoproterenol (1 μmol/L) plus the β_1AR-selective antagonist CGP20712A (300 nmol/L; online-only Data Supplement).[21,29]

In Vitro Takotsubo Cardiomyopathy Model

Freshly isolated rat ventricular cardiomyocytes were perfused with epinephrine (100 nmol/L) for 20 minutes followed by washout (10 minutes). A subgroup of cells were preincubated with PTX (1.5 μg/mL) for 3 hours at 35°C.

βAR Radioligand Binding Assay

Cell membranes, prepared from apical- and basal-derived adult rat cardiomyocytes, were incubated for 2 hours at room temperature in assay buffer (50 mmol/L Tris, 5 mmol/L $MgCl_2$; pH 7.4), with 0.1 to 10 nmol/L of the nonselective βAR radioligand [^{125}I]-cyanopindolol ([^{125}I]-CYP; Amersham) and increasing concentrations of the selective β_2AR antagonist ICI-118,551 (1×10^{-11} to 1×10^{-2} mol/L). Nonspecific binding was determined in the presence of 10 μmol/L of the nonselective βAR antagonist propranolol.

Fluorescence Resonance Energy Transfer–Mediated cAMP Assay

Förster resonance energy transfer (FRET) studies in EPAC (exchange protein directly activated by cAMP)-cAMPs expressing apical and basal ventricular cardiomyocytes were performed as described previously.[31] Whole-cell epinephrine-stimulated β_2AR-mediated cAMP transients were recorded. A subgroup of cells were preincubated with PTX (1.5 μg/mL) for 3 hours at 35°C.

Human Tissue Samples and Cardiomyocyte Isolation

Left or right ventricular tissues were obtained from failing human hearts at the time of heart transplantation; procedures for collecting human heart tissues conformed to the ethics committee requirements of the Royal Brompton and Harefield Hospital. Written informed consent was provided by all patients. The investigation conformed to the principles outlined in the Declaration of Helsinki. Single human ventricular myocytes were isolated from explanted failing human hearts by a standard enzymatic technique as described previously.[32]

Cardiomyocyte Contractility Studies

The online-only Data Supplement contains a detailed Methods section.

Statistical Methods

Results are shown as mean±SEM. Differences between cell responses for different treatments were determined by paired or unpaired Student *t* tests for 2 conditions or by 1-way analysis of variance (ANOVA) for ≥2 conditions. Concentration-response curves were compared by repeated-measures ANOVA. Comparisons between responses to bolus epinephrine and norepinephrine or with or without PTX, β-blockers, or levosimendan were performed by repeated-measures ANOVA with time as a second factor. For individual time points after bolus, significant changes in fractional shortening compared with the preadministration period are indicated; *P* values less significant than $P<0.01$ are not shown because of the multiple comparisons. Differences in mortality were analyzed with the χ^2 test, with Fisher exact test for small numbers. *P* values of ≤0.05 are taken as significant.

Results

High-Dose Epinephrine Injection Recapitulates Takotsubo Cardiomyopathy

High serum epinephrine levels are a common feature in Takotsubo cardiomyopathy patients, which suggests a mechanistic link. We developed a model of Takotsubo cardiomyopathy in which an anesthetized rat received an intravenous (jugular) bolus of epinephrine 4.3×10^{-8} mol/100 g (equivalent to ≈5 mg in an adult human). Intravenous bolus delivery was selected to mimic the human physiological response to sudden high stress. Initial dose-response curves determined the highest catecholamine dose without excessive mortality (online-only Data Supplement Figure I). Epinephrine bolus triggered a rapid hypertensive response with reflex bradycardia within seconds of administration, which stabilized to normotension after several minutes (online-only Data Supplement Figure II) and was associated with an initial global increase in left ventricular contractility (Figure 1); however, this declined and yielded a marked decrease in cardiac contraction, initiated at 15 minutes and reaching a nadir between 20 and 25 minutes. Contraction normalized within an hour. One defining characteristic of Takotsubo cardiomyopathy is the apical and midventricular localization of dysfunction, and this was clearly reproduced in the present model (Figure 1) and was confirmed by cardiac magnetic resonance imaging (online-only Data Supplement III and Movie I).

Apical Hypokinesia Is Epinephrine Specific

We and others have previously reported that epinephrine or isoproterenol at high concentrations can switch the β_2AR from positively inotropic G_s to negatively inotropic G_i coupling,[17,22,33] whereas norepinephrine cannot.[16] We found that equivalent high-dose intravenous norepinephrine did not generate the negative effect observed after epinephrine bolus (Figures 1A through 1C), and concentration-response curves (online-only Data Supplement Figure I) confirmed that no concentration of norepinephrine was negatively inotropic. Changes in heart rate and systemic arterial blood pressure did not differ between epinephrine and norepinephrine, which indicates appropriate matching of effective concentrations (online-only Data Supple-

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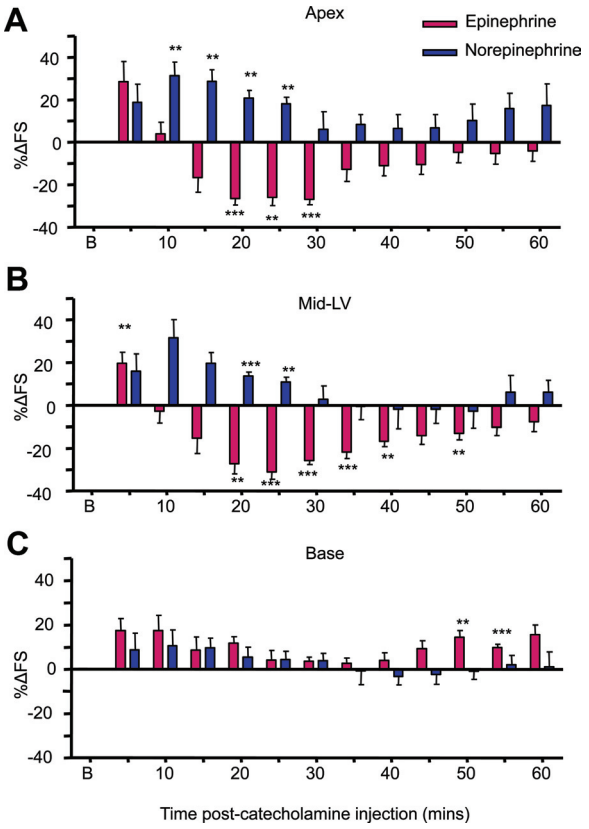

Figure 1. Takotsubo cardiomyopathy is epinephrine specific. Effects of epinephrine 4.28×10^{-8} mol/100 g (red bars) and norepinephrine 1.43×10^{-7} mol/100 g (blue bars) on apical (**A**), mid left ventricular (**B**), and basal (**C**) myocardium contractility. Values are expressed as mean percentage change in left ventricular fractional shortening (%ΔFS) from baseline (untreated) levels ±SEM at each 5-minute time point after injection. n=6, epinephrine; n=6, norepinephrine. **$P<0.01$, ***$P<0.001$, ****$P<0.0001$ vs baseline FS=0. B indicates baseline; LV, left ventricular. Repeated-measures ANOVA, epinephrine vs norepinephrine: $P<0.001$, apex; $P<0.001$, mid LV; P=NS, base; time: $P<0.001$, apex; $P<0.001$, mid LV; $P<0.05$, base.

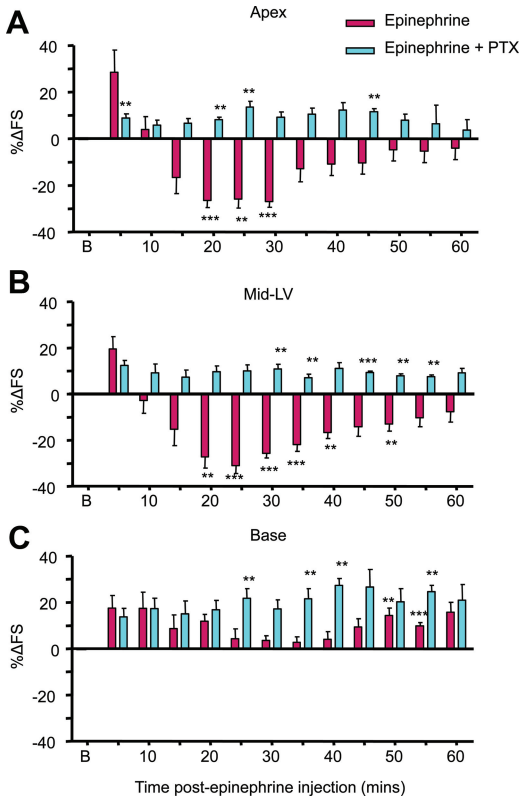

Figure 2. In vivo Takotsubo cardiomyopathy model and prevention by pertussis toxin (PTX) pretreatment. Contractile responses after an intravenous bolus injection of epinephrine (4.28×10^{-8} mol/100 g; red bars) on left ventricular apical (**A**), mid left ventricular (**B**), and basal (**C**) myocardium. Values are expressed as mean percentage change in left ventricular fractional shortening (%ΔFS) from baseline (untreated) levels ±SEM at each 5-minute time point after injection. Blue bars show time-matched inotropic responses of apical, mid left ventricular (MLV), and basal myocardium in PTX (25 μg/kg)–pretreated animals after equivalent intravenous epinephrine bolus, with loss of apical and MLV hypokinesis. n=6, control epinephrine; n=5, epinephrine+PTX. **$P<0.01$, ***$P<0.001$ vs baseline FS=0. B indicates baseline. Repeated-measures ANOVA (epinephrine vs epinephrine+PTX): $P<0.001$, apex; $P<0.01$, MLV; $P<0.05$, base; time: $P<0.001$, apex; $P<0.001$, MLV; $P<0.001$, base.

ment Figure II). Lack of negative effect of norepinephrine additionally eliminates either myocardial β_1AR- or α_1AR-mediated vasoconstriction as the principal mediator of the epinephrine-stimulated negative inotropic effect.

Epinephrine-Induced Apical Hypokinesia Is G_i Dependent

We used PTX to inhibit G_i by in vivo pretreatment of the rats 3 days before the intravenous epinephrine challenge. In vitro challenge of isolated cardiomyocytes from these hearts with carbachol (after βAR stimulation) was used to verify inhibition of G_i effects (not shown). The negative effect of epinephrine was completely abolished by PTX (Figures 2A through 2C), which provides strong evidence for a G_i-dependent mechanism of action. Apical and midventricular regions switched completely to give an increase in contraction, and even basal hypercontractility was significantly enhanced. PTX pretreatment did not alter baseline function, the systemic arterial pressure response to epinephrine (online-only Data Supplement Figure III), or time-matched responses after control saline bolus (online-only Data Supplement Figures IV and V). PTX pretreatment reduced the vagally mediated reflex bradycardia during the first minutes after epinephrine injection (online-only Data Supplement Figure IIE). However, systemic vagal blockade with atropine pretreatment failed to prevent epinephrine-induced hypokinesia as observed with PTX pretreatment and significantly increased mortality caused by cardiogenic shock (online-only Data Supplement Figure VI). This excluded systemic vagal inhibition as the explanation for the PTX-mediated prevention of apical hypokinesia.

We also developed an in vitro model in which isolated rat ventricular cardiomyocytes were treated for 20 minutes with epinephrine. These cells showed a decreased positive inotropic response to a subsequent β_2AR challenge (Figure 3A; online-only Data Supplement VII). Maximum responses to high calcium were unchanged, which indicates that overall cellular and contractile function was not compromised (online-only Data Supplement Figure VIII). The depression of β_2AR response after epinephrine pretreatment observed in this in vitro model was completely prevented (and the

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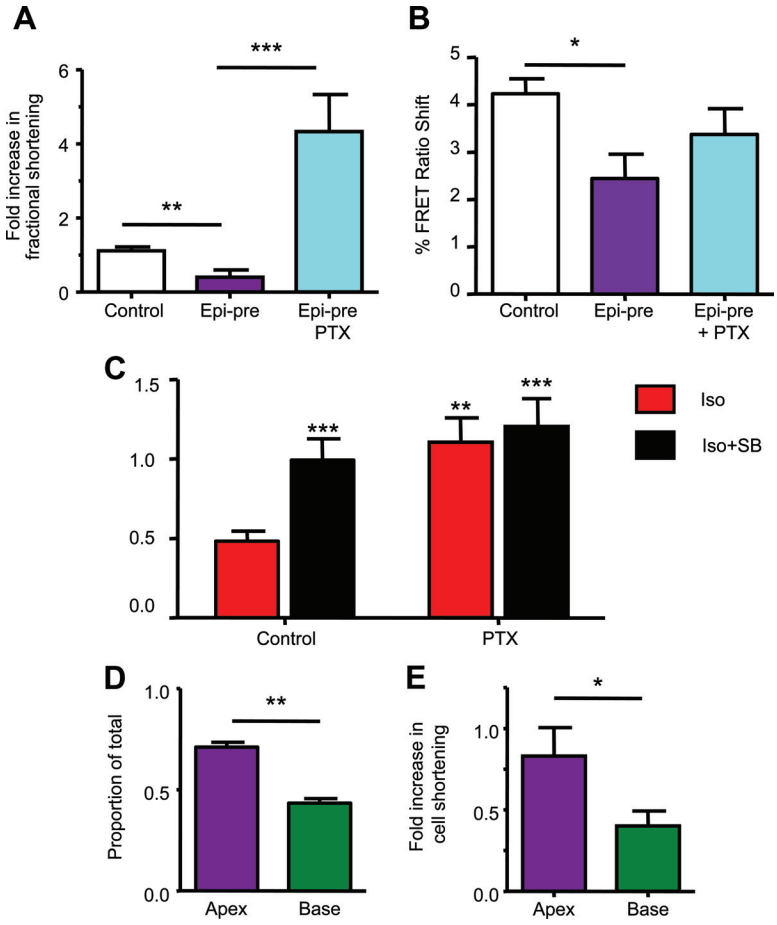

Figure 3. In vitro Takotsubo cardiomyopathy model induced by high-dose epinephrine exposure. Effect of 20-minute pretreatment with epinephrine (Epi-pre; 100 nmol/L) followed by 10-minute wash on subsequent β_2-adrenergic receptor (β_2AR) contractile (**A**) and cAMP (**B**) responses with G_i (pertussis toxin [PTX] sensitive) component. **A**, Contraction amplitude in isolated rat ventricular myocytes. Peak fold increase over basal: Control, n=15; Epi-pretreated alone, n=15; Epi-pretreated+PTX, n=7. **B**, Whole-cell cAMP levels, measured with an EPAC2-FRET (Förster resonance energy transfer) sensor. Control, n=40; Epi-pretreated alone, n=10; Epi-pretreated+PTX, n=9. *P<0.05, **P<0.01, ***P<0.001, 1-way ANOVA Kruskal-Wallis test. **C**, β_2AR-mediated inotropic response to isoproterenol 1 μmol/L (ISO) in the presence of the β_1AR blocker CGP20712A (300 nmol/L): Peak fold increase over basal in control (n=13) or PTX-treated rat ventricular myocytes (n=13), in the presence and absence of SB20380 2.5 μmol/L (SB). **P<0.01, ***P<0.001 vs control, unpaired t test. **D** and **E**, Apically derived cardiomyocytes demonstrate increased β_2AR levels and responses. **D**, Proportion of β_2ARs with respect to total βAR radioligand binding in ventricular myocytes from the apex and base of normal rat heart. n=4 preparations. **P<0.01 vs base, paired t test. **E**, Apical cardiomyocytes (purple bars) showed a larger increase in percentage cell shortening through β_2AR than basal cardiomyocytes (green bars). Fold increase in shortening with isoproterenol 1 μmol/L plus CGP20712A 300 nmol/L. *P<0.05 apex vs base, paired t test. Base, n=13 cells; apex, n=13 cells; n=13 animals.

response became higher than control) after PTX treatment (Figure 3A; online-only Data Supplement VII). Notably, measurement of cAMP under the same conditions showed much less marked changes: PTX treatment increased contraction 11-fold without a significant increase in cAMP levels (Figure 3B). This implies a parallel negative inotropic pathway activated through G_i. Because p38 MAPK has been shown to be both G_i-dependent and negatively inotropic in rat ventricular myocytes, we compared treatment with PTX and a p38 MAPK inhibitor (Figure 3C). Both were able to increase β_2AR responses to a similar degree, and the effects of the 2 were not additive.

Apical Ventricular Cardiomyocytes Have Higher β_2AR Density and β_2AR-Mediated Contractile Responses Than Basal Cardiomyocytes

We have hypothesized that the increased apical sensitivity observed in Takotsubo cardiomyopathy patients and our model is caused by a greater proportion of β_2ARs relative to β_1ARs in the apex,[8] because the greater concentration of sympathetic innervation in the base of the heart[13] is counterbalanced by increased apical βAR functional responses to circulating catecholamines.[9–12] Using a radioligand binding-displacement assay to directly quantify the β_2 to β_1AR ratio, we found that apical cardiomyocytes demonstrated an increased β_2 to β_1AR ratio (Figure 3D). The functional consequences of a higher β_2 to β_1AR ratio were studied and confirmed greater β_2AR-specific contractile responses in apical ventricular cardiomyocytes than in paired basal cardiomyocytes isolated from the same heart (Figure 3E). β_2AR-dependent and maximal cAMP responses demonstrated no difference between apical and basal cardiomyocytes (online-only Data Supplement Figure IX) and therefore could not explain the observed gradient and contractile response.

Epinephrine-Induced β_2AR-G_i Signaling Is Cardioprotective

Because β_2AR-G_i is widely reported to be antiapoptotic and cardioprotective,[34–36] we hypothesized that blocking β_2AR-G_i signaling might increase the cardiotoxic effects of high epinephrine levels via uninhibited β_1AR-G_s and β_2AR-G_s signaling. In the rat Takotsubo model in vivo, epinephrine-induced mortality was increased significantly by prior selective β_2AR blockade with ICI-118,551 (at concentrations insufficient to activate G_i) or p38 MAPK inhibition with SB203580 (Figure 4A). Death often occurred within 5 to 10 minutes and was caused by cardiogenic shock and hypokinesia rather than primary ventricular fibrillation. In vitro, isoproterenol increased cell death in cultured myocytes, an effect largely inhibited by β_1AR blockade (Figure 4B), whereas overexpression of β_2AR (Figure 4B) or G_i (Figure 4C) protected against catecholamine-induced cell death. β_2AR switching from G_s to G_i coupling is thought to be enhanced after strong βAR-G_s activation, mediated by cAMP-dependent protein kinase (PKA).[18] Overexpression of a β_2AR construct in which PKA sites had been mutated to prevent phosphorylation not only

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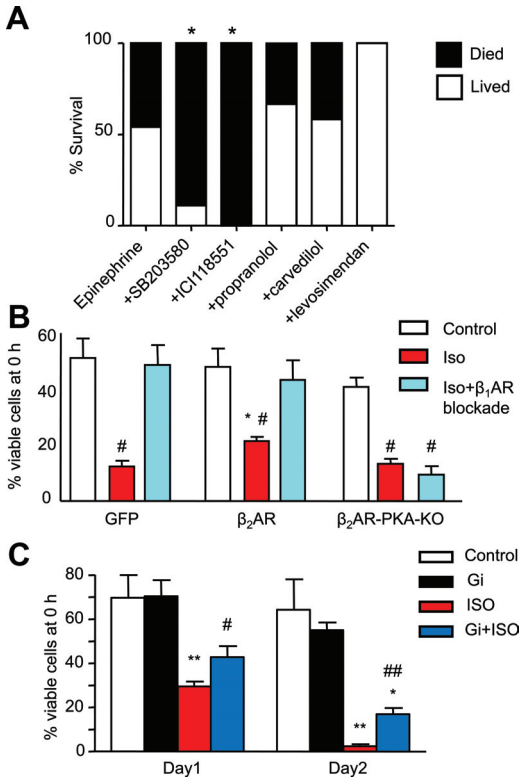

Figure 4. Epinephrine-mediated β_2-adrenergic receptor (β_2AR)–G_i signaling is cardioprotective. **A**, Mortality with in vivo bolus epinephrine (4.28×10^{-8} mol/100 g) in the absence (n=14) or presence of SB203580 0.1 to 10 μg/kg (n=9), ICI-118,551 1 μg/kg (n=5), propranolol 1.43×10^{-11} mol/100 g (n=9), carvedilol 1.43×10^{-11} mol/100 g (n=12), and levosimendan 4.7 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (n=5). *P<0.05 vs epinephrine alone, Fisher exact test. **B**, Survival of adult rat ventricular myocytes (percent remaining at 48 hours compared with time 0) after exposure to isoproterenol (ISO) 1 μmol/L in the presence (blue bars) and absence (red bars) of the β_1AR blocker CGP20712A (300 nmol/L) compared with untreated controls (white bars). Myocytes were transduced with adenoviral vectors with green fluorescent protein (GFP; control), the wild-type β_2AR, and β_2AR with mutations at the protein kinase A (PKA) phosphorylation sites 261, 262, 345, and 346 S/A (β_2AR-PKA-KO) to prevent switching to G_i. n=6. #P<0.05 vs control/GFP, *P<0.05 vs GFP+ISO (1-way ANOVA). **C**, Effect of G_i expression on ISO-induced myocyte toxicity over 48 hours in culture. Myocytes were transduced with adenoviral vectors with GFP (control) or G_i-GFP (G_i) at day 0. n=6 preparations. *P<0.05, **P<0.01 vs respective control; #P<0.05, ##P<0.01 vs ISO alone (1-way ANOVA).

failed to protect but produced β_1AR-independent cell death (Figure 4B). This mutant was also unable to support β_2AR-dependent negative inotropism, in contrast to wild-type β_2AR (online-only Data Supplement Figure X).

β-Blockers That Activate β_2AR-G_i Do Not Rescue and May Worsen Established Apical Hypokinesia

In the previous section, we noted that pretreatment with a specific β_2AR blocker before the epinephrine bolus did not appear to be a therapeutically useful maneuver. We also predicted that clinically used β-blockers that activate β_2AR-G_i might exacerbate the epinephrine-induced negative inotropic effect. The G_i-dependent negative effect of β-blockers is most readily seen in myocytes from failing human hearts (in which G_i is increased[29]); we selected compounds that had either strong (propranolol) or modest (carvedilol) effects on these cells (Figure 5A). Figures 5B and 5C show the effect of the 2 blockers added 15 minutes after epinephrine in the in vivo model, when peak negative responses were developing. Propranolol, with higher β_2AR-G_i agonism, significantly enhanced and prolonged the negative effects of epinephrine at both apex and base (Figure 5B), whereas carvedilol, with less pronounced β_2AR-G_i agonism, had little effect on the apex but converted the base from positive to significant negative responses (Figure 5C). In contrast, the β_1AR-selective blocker bisoprolol reduced the positive effect of epinephrine at the base but did not convert it to a significant negative response; there was no effect on the apical epinephrine response (online-only Data Supplement Figure XI). These data support our hypothesis of synergistic effects of epinephrine with propranolol (and to a minor extent carvedilol) on β_2AR-G_i signaling. Although the negative inotropic of epinephrine was enhanced, there was no increase in mortality with the addition of propranolol or carvedilol (Figure 4A).

Levosimendan Reverses Epinephrine-Induced Apical Dysfunction Without Increased Mortality

Levosimendan was selected for comparison because it is an inotrope with a cAMP-independent mechanism of action, which increases myofilament calcium sensitivity.[37] Global cardiac contraction in untreated hearts was increased with infusion of this compound (data not shown). In contrast to other agents, application of levosimendan at the point at which epinephrine negative effects were beginning was effective in preventing further decline in cardiac function (Figure 6). This contractile benefit and rescue occurred with no deaths in the epinephrine-treated group (Figure 4A).

Discussion

Takotsubo cardiomyopathy is an increasingly recognized acute cardiac syndrome in the modern era of early access to diagnostic coronary angiography.[1–3] As a cardiac response to extreme stress levels, it carries a relatively good prognosis but has the intriguing feature of regional (apical) hypokinesia, which is counterintuitive in relation to the systemic nature of the trigger and the evolutionary drive for increased cardiac output during "flight-or-fight" responses. We have developed a rat model that mimics the clinical features, with acute, reversible apical and midventricular myocardial hypokinesia but preserved or enhanced basal contractility (Figure 1). A rapid high-dose intravenous epinephrine bolus, designed to mimic the serum catecholamine response to acute stress compared with the traditional infusion protocols, recapitulated the classic clinical findings, whereas the equivalent norepinephrine bolus did not (Figure 1). This implied the mechanism is epinephrine specific and confirms the observation that dysfunction is not typically observed in the region with the highest density of norepinephrine-releasing sympathetic nerve terminals.[13]

We further investigated this concept of apical-basal gradients of catecholamine responsiveness to βAR subtype and demonstrated that apical ventricular cardiomyocytes have a higher β_2AR density and a greater β_2AR-induced sensitivity than basal cardiomyocytes isolated from the same heart (Figures 3D and 3E). The inability of norepinephrine at equivalent (and higher) doses to initiate acute apical dysfunc-

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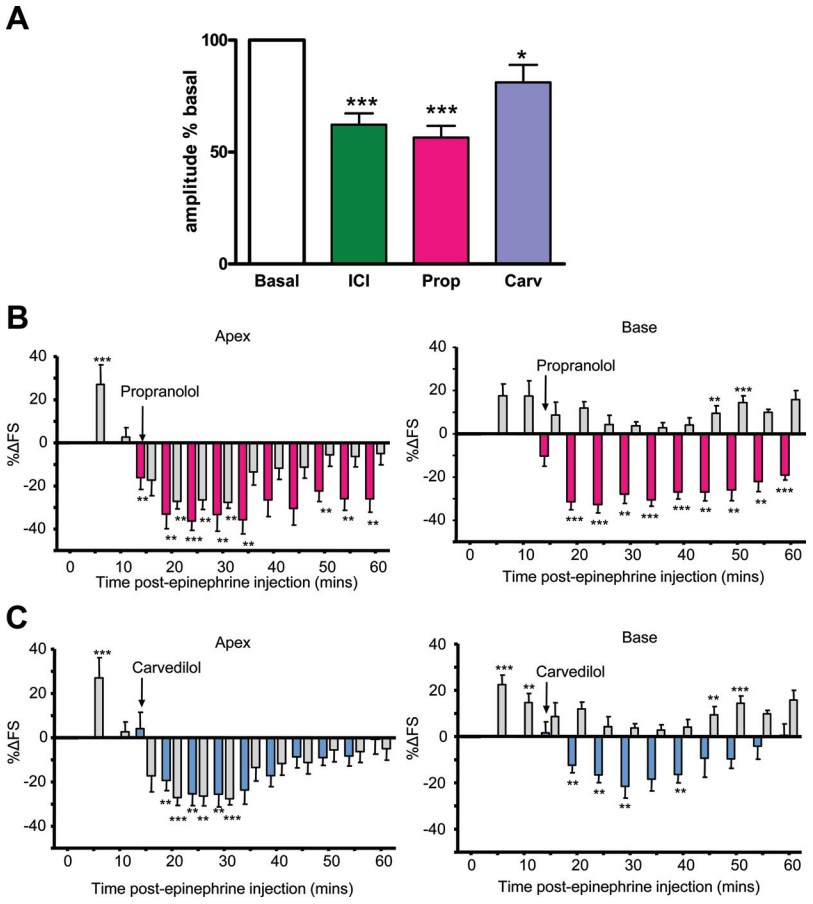

Figure 5. Agonist-independent negative inotropic effect of β-blockers and potentiation of Takotsubo cardiomyopathy. **A**, Negative inotropic effect of β-adrenergic receptor (βAR) blockers on contraction of ventricular myocytes from failing human heart. Contraction amplitude relative to basal (open bar) for ICI-118,551 (ICI; 3 μmol/L, n=21), propranolol (Prop; 5 μmol/L, n=9) and carvedilol (Carv; 3 μmol/L, n=24). *$P<0.05$, ***$P<0.001$ vs 100% (1-way ANOVA). **B** and **C**, The β-blockers propranolol (**B**) and carvedilol (**C**; both 1.43×10^{-11} mol/100 g IV) either enhanced or failed to prevent the negative inotropic effects of epinephrine (4.28×10^{-8} mol/100 g IV) at the apex and reversed the positive effects of epinephrine at the base in the in vivo rat model. Values are expressed as mean percentage change in left ventricular fractional shortening (FS) from baseline (untreated) levels ±SEM at each 5-minute point after intravenous injection. n=6, epi; n=6, epinephrine+propranolol; n=7, epinephrine+carvedilol. **$P<0.01$, ***$P<0.001$, ****$P<0.0001$ vs baseline FS=0. Repeated-measures ANOVA: Epinephrine vs epinephrine+propranolol: Apex $P=0.05$, base $P<0.01$; time: apex $P<0.001$, base $P<0.001$. Epinephrine vs epinephrine+carvedilol: apex P=NS, base $P<0.001$. Time: apex $P<0.001$, base $P<0.001$.

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tion excludes coronary vasospasm or β_1AR-mediated signaling as a primary effector (Figure 1). This is in agreement with clinical observations that the apical dysfunction in Takotsubo cardiomyopathy extends beyond the territory of a single coronary bed.[1–3,8] Also supporting the predominance of a cardiomyocyte-based explanation rather than a vascular one is the ability of the present in vitro cardiomyocyte model to reproduce a number of the key in vivo observations (Figure 3), as well as the matched responses of heart rate and blood pressure between the epinephrine and norepinephrine cohorts.

Norepinephrine also differs in that it does not couple β_1ARs or β_2ARs to G_i signaling, whereas epinephrine at high concentrations produces a β_2AR G_s-to-G_i switch. β_2AR-G_i coupling has been reported in a number of experimental models, including β_2AR and G_i overexpression, and importantly in chronic heart failure, in which G_i levels are increased.[29] β_2AR-G_i coupling occurs via a process termed *stimulus/ligand-directed trafficking* or *biased agonism*. Other agonists, such as high-dose isoproterenol, also produce this switch, and we note a study in which isoproterenol infusion over 2 weeks also produced a specific apical contraction defect.[9] The key role of G_i in the cardiodepression was shown by the ability of PTX to convert apical responses to epinephrine from negative to positive (Figure 2). The response of basal myocardium was also increased, which implies that β_2AR-G_i was operational even in this region despite the β_1AR predominance. Nonclassic examples of Takotsubo cardiomyopathy have been observed in which the base or mid left ventricle is affected,[38] and this may reflect individual patterns of β_2AR expression. The in vivo observations were supported by those in isolated cells. In untreated apical myocytes, positive inotropic responses to β_2AR stimulation were enhanced by PTX (Figure 3C; online-only Data Supplement Figure VII; and as reported previously[39]). In myocytes pretreated with epinephrine, PTX was able to rescue and further enhance the depressed β_2AR-mediated positive responses (Figure 3A; online-only Data Supplement Figure VII). cAMP responses were decreased modestly in pretreated myocytes (Figure 3B), although they were less affected than contractile responses. However, PTX was able to rescue contractile responses with no significant effect on cAMP (Figure 3B), which implies the existence of a separate, negatively inotropic G_i-dependent pathway. Inhibition of p38 MAPK produced similar and nonadditive effects to PTX, consistent with the suggested role for this pathway as a G_i-dependent, negatively inotropic modulator.

The epinephrine-dependent β_2AR-G_i–mediated negative inotropism requires a preceding high β_1AR-G_s activation to initiate cAMP-dependent PKA and GRK phosphorylation of the β_2AR.[18,40] This implies that although norepinephrine does not directly couple receptors to G_i, the rise in cAMP it produces will predispose the β_2AR to traffic to G_i on subsequent epinephrine binding. Here, we demonstrate that PKA-mediated β_2AR phosphorylation is critical for G_i coupling, because deleting the phosphorylation sites prevented both negative inotropism and cardioprotection attributable to

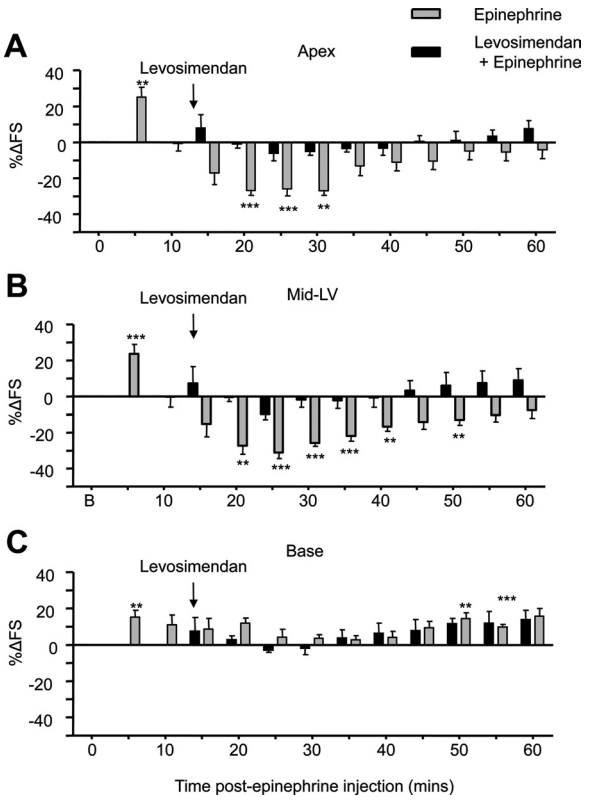

Figure 6. Levosimendan rescues the Takotsubo cardiomyopathy model. Effects of levosimendan 0.28 mg · kg^{-1} · h^{-1} (4.7 μg · kg^{-1} · min^{-1}) intravenous infusion (black bars) on inotropic responses of apical (**A**), mid left ventricular (**B**), and basal (**C**) myocardium contractility after epinephrine 4.26×10^{-8} mol/100 g IV compared with epinephrine alone (gray bars). Values are expressed as mean percentage change in left ventricular (LV) fractional shortening (FS) from baseline ±SEM at each 5-minute time point after injection. n=6, epinephrine; n=5, levosimendan+epinephrine. **P<0.01, ***P<0.001, ****P<0.0001 vs baseline FS=0. Repeated-measures ANOVA: Epinephrine vs epinephrine+levosimendan: apex P<0.01; mid LV, P<0.01; base P=NS. Time: apex P<0.001; mid LV P<0.001; base P<0.001.

β_2AR-G$_i$ coupling (Figure 4B; online-only Data Supplement Figure X). This also explains the reversibility of the Takotsubo cardiomyopathy syndrome. As the serum epinephrine levels fall, β_2AR dephosphorylation, or internalization and replacement with de novo unphosphorylated β_2ARs, reduces the β_2AR-G$_i$ stimulus trafficking and restores normal contractile function in the surviving cardiomyocytes. Studies in model cell systems overexpressing fluorescently labeled β_2AR have demonstrated the dependence of both PKA- and GRK-mediated β_2AR phosphorylation for β_2AR internalization from the surface membrane and recycling to different surface microdomains.[19] Interestingly, they have also demonstrated the epinephrine-specific dependence of this trafficking.[40] This is relevant to patients with Takotsubo cardiomyopathy, because to date, there has been a failure to identify any associated polymorphisms in the α_1ARs, β_1AR, or β_2ARs,[41] but one study, albeit with small patient numbers, found an increased prevalence of the GRK polymorphism L41Q in the Takotsubo cardiomyopathy patient cohort compared with healthy matched control subjects.[20] This gain-of-function mutation, previously referred to as genetic β-blockade,[42] confers reduced responsiveness to βAR agonists and improves prognosis in the population carrying this polymorphism,[42] both conceivably consistent with enhanced myocardial β_2AR-G$_i$ coupling.

Although the final outcome for the patient with Takotsubo cardiomyopathy is generally good, they have been through an acute cardiac event that requires hospitalization, and there is a significant incidence of cardiogenic shock (≈4%), malignant ventricular arrhythmias (1%–2%), and death (1%–1.5%). It therefore seemed reasonable to try to block the depression of contraction with either a specific β_2AR antagonist or a p38 MAPK inhibitor; however, the marked increase in mortality produced by this maneuver gave a clear indication that this was a counterproductive strategy (Figure 4A). The rapidity of death, usually within 5 to 10 minutes and always within 45 minutes, made it unlikely that apoptosis was the underlying mechanism. β_2AR or G$_i$ has been implicated in suppression of arrhythmias,[43] and β_2AR variants have been implicated in sudden cardiac death.[44] β_2AR knockout mice went into cardiogenic shock after doxorubicin administration, through a β_1AR-related mechanism,[45] and β_2AR/G$_i$ mechanisms have been implicated in postischemic stunning.[46] All of these are potential mechanisms that could underlie acute mortality. We suggest that the enhanced β_2AR-G$_i$ coupling initiated by high epinephrine levels is protective because it dampens the effects of toxic βAR-G$_s$ coupling, which would be fatal if left unchecked.

Few β-blockers are pure neutral antagonists, with most having some other effect, such as partial agonism (intrinsic sympathomimetic activity), inverse agonism (reduction in activity of constitutively active receptors), or biased agonism (ligand-directed trafficking to other pathways). It has been amply demonstrated that β_2AR blockers can activate other signaling pathways, both G-protein and non–G-protein dependent.[47] We were first alerted to the possibility that the cardiodepressant effects in Takotsubo cardiomyopathy were β_2AR-G$_i$ dependent by their similarity to the β_2AR-G$_i$–mediated effect of β-blockers on myocytes from failing human heart.[29] We therefore hypothesized that β-blockers with strong β_2AR-G$_i$ agonism would synergize with the negative inotropic effect of epinephrine. Propranolol, a particularly cardiodepressant agent, markedly enhanced and prolonged the negative phase when given after the epinephrine bolus in the present in vivo model (Figure 5B). In support of the hypothesis of additive negative inotropic effects of propranolol and epinephrine, we note a recent report that an acute dilated cardiomyopathy was precipitated in a patient with pheochromocytoma after taking propranolol for migraine.[48] Carvedilol had a more modest effect, reversing basal hypercontractility while having a neutral effect on apical hypokinesia (Figure 5C). Carvedilol and propranolol are also able to produce biased agonism through G$_{\beta\gamma}$ mechanisms,[49] which would be PTX sensitive. Although possibly exacerbating the syndrome, carvedilol could be useful in the treatment of the minority of Takotsubo cardiomyopathy patients with severe left ventricular outflow tract obstruction secondary to basal hypercontractility. Neither blocker had a deleterious effect on mortality in the Takotsubo cardiomyopathy model. Bisoprolol, which is predominantly β_1AR selective, did not reproduce these effects to synergize with epinephrine.

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We considered the implications for treating Takotsubo cardiomyopathy and for heart failure therapy more generally. For the patient with Takotsubo cardiomyopathy, strategies to raise cAMP (catecholaminergic inotropes or phosphodiesterase inhibitors) would clearly be contraindicated. Indeed, dobutamine administered for stress echocardiography testing has precipitated Takotsubo cardiomyopathy.[7] A cAMP-independent inotrope, levosimendan, was effective in reversing the negative inotropic effect of epinephrine, and rescue occurred without increased (and a trend to decreased) mortality (Figures 4A and 6). We suggest that this is likely to be a safe supporting and bridging strategy for the sickest patients with cardiogenic shock until spontaneous recovery occurs, and preliminary clinical reports support this view.[50,51] At the higher doses, levosimendan can inhibit phosphodiesterases[37] and increase cAMP, and thus, we only would recommend the lower (nonvasodilatory) doses. The value of nonselective β-blockers, which may also act as agonists at β_2AR-G_i, is more difficult to predict, because they may amplify both the negative inotropic and the protective effects of epinephrine. It could further be suggested that the beneficial effects of β-blockers in heart failure has taken serendipitous advantage of cardioprotective β_2AR-G_i biased agonism. If those 2 effects could be modulated separately, this might point the way for an improved design of future β-blockers by their selection for cardioprotection through β_2AR-G_i biased agonism.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Takotsubo cardiomyopathy is an increasingly recognized clinical syndrome characterized by severe acute (but reversible) apical ventricular dysfunction, ST elevation, and unobstructed coronary arteries. Physical or emotional stress is a frequent precipitant, as well as iatrogenic catecholamine exposure. We generated a model of Takotsubo cardiomyopathy using a single high bolus dose of epinephrine in anesthetized rats, which reproduced the reversible apical hypocontractility. We explored the subcellular mechanisms and identified that high-dose epinephrine activated a switch of β_2-adrenergic receptor (β_2AR) coupling from the positively inotropic G_s-cAMP to a negatively inotropic G_i signaling pathway. Norepinephrine, which does not activate this G_s/G_i switch, did not precipitate apical dysfunction. Proportionately higher β_2AR numbers at the apex produced the apical-basal gradient in contractility. Prevention of the switch increased sudden death in this model, which is consistent with the protective antiarrhythmic and antiapoptotic nature of G_i signaling. We screened various β-blockers (given after apical dysfunction was established) using the model and noted that propranolol, which also switches β_2AR to the G_i pathway, exacerbated contractile dysfunction without increasing mortality. Carvedilol had similar but less pronounced effects, and bisoprolol did not affect the response. Avoiding the βAR-cAMP axis with the calcium myofilament sensitizer levosimendan, we were able to completely rescue the acute Takotsubo syndrome, with 100% survival. This model suggests that an epinephrine-induced β_2AR G_s/G_i switch is responsible for the acute apical dysfunction in Takotsubo cardiomyopathy but that it may also confer an element of protection against damage by high catecholamines.

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