Chapter 4

Adrenaline and Stress-Induced Cardiomyopathies: Three Competing Hypotheses for Mechanism(s) of Action

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Abstract

Stress-induced cardiomyopathies such as *Tako-Tsubo Syndrome* (also known as *"Broken-Heart Syndrome"*) primarily affect post-menopausal women who have experienced a sudden emotional shock. Clinical presentation includes symptoms mimicking a myocardial infarction (severe chest pain and S-T elevation on EKG), but do not show significant occlusion of the coronary arteries. Instead, patients display left ventricular (LV) dysfunction characterized by hypo- or a-kinetic regions that appear to "balloon-out", particularly in the region near the apex, and thus is sometimes referred to as *"Apical-Ballooning Syndrome"*. Some patients have been reported with high circulating levels of adrenaline, and symptoms have been effectively managed in many cases by treatment with beta-adrenergic receptor blockers. It is not entirely clear, however, why only specific regions of the LV were affected in these patients, nor is

it understood why postmenopausal women are so susceptible relative to the rest of the population. With respect to the first question, we will review three competing, though not necessarily mutually exclusive, hypotheses to explain how adrenaline plays a key role in precipitating stress-induced cardiomyopathies. The first of these is a vascular microspasm hypothesis which focuses on stress-induced changes in the coronary microvasculature feeding the LV, leading to microspasms, interrupted regional blood-flow, and corresponding myocardial dysfunction in affected areas of the LV (Sato et al., 1990 and Dote et al., *J Cardiol* 1991;21:203) [6, 7]. The second hypothesis will be referred to as the differential β-receptor expression hypothesis, which postulates there is a higher density of β-adrenergic, (especially β_2-adrenergic) receptors in the apical region of the LV compared to other regions, thereby making it more sensitive to adrenergic overload and myocardial stunning due to agonist-mediated switch from Gs to Gi coupling of the β_2-adrenergic receptors in this region relative to other regions of the heart (Lyon et al., *Nature Clin Pract Cardiovasc Med* 2008;5:22) [10]. A third hypothesis focuses on differential local production of adrenergic hormones within the left myocardium itself (Kume et al., *Circ J* 2008; 72:106 and Osuala et al., *PLoS One* 2011;8:e22811) [1, 3]. From this third hypothesis, selective myocardial stunning in the LV results from local overload of adrenergic stimulation due to autocrine/paracrine actions of adrenaline (epinephrine) and noradrenaline (norepinephrine) in addition to sympathetic stimulation and circulating catecholamines in periods of stress. The evidence for each hypothesis is critically evaluated, with discussion of potential future directions for work in this field in relation to the role of gender (sex), age, and menopausal status.

Introduction

The impacts of emotional stress on health have been generally recognized since ancient times, but only in relatively recent years have scientists and clinicians started to gain an understanding of the pathophysiological mechanisms linking emotional stress to specific dysfunctions within the cardiovascular system. In contrast, biological manifestations of physical stress are much more extensively characterized, and broadly include hypertension, ischemic heart disease (including myocardial infarction and its consequences), myocarditis, cardiomyopathies, heart failure, arrhythmias, and sudden cardiac death [11]. While emotional stress may also contribute to these conditions, the specific pathophysiologic manifestations of emotional stress are only beginning to emerge.

Takotsubo Syndrome is a prototypical stress-induced cardiomyopathy. The first clinical cases of this stress cardiomyopathy were reported in Japan in 1990 [7]. Early reports described a transient hypokinesis or akinesis in the apex of the LV in patients suffering from acute emotional stress [12-14]. The unusual shape of the heart in these patients bore a striking resemblance to a Japanese octopus trap, and hence, it was named "takotsubo", meaning octopus trap in Japanese [6, 7, 13] (Figure 1). Takotsubo Syndrome is also sometimes referred to as "Broken Heart Syndrome" (due to the emotional stress connection), "Ampulla Cardiomyopathy" (due to the peculiar shape of the heart), or "Apical Ballooning Syndrome" (to describe one of the most pronounced clinical features commonly observed in these patients) [14]. Takotsubo Syndrome is also referred to as a "Stress-Induced Cardiomyopathy" or more simply, "Stress Cardiomyopathy". These terms have become essentially interchangeable when describing this syndrome [15]. For the purpose of this review, we will use the historical "Takotsubo Cardiomyopathy (TTC)" designation as the prototype clinical form of stress-induced cardiomyopathies. It is important to note, however, that stress induction for TTC need not be exclusively emotional or psychological, but can also be precipitated by physical stressors such as pharmacological challenge with adrenaline and other adrenergic agonists [16, 17], complications from other diseases (e.g., pheochromocytoma) [18, 19], physical trauma from injury or surgery, and a wide variety of other physical stressors [20, 21].
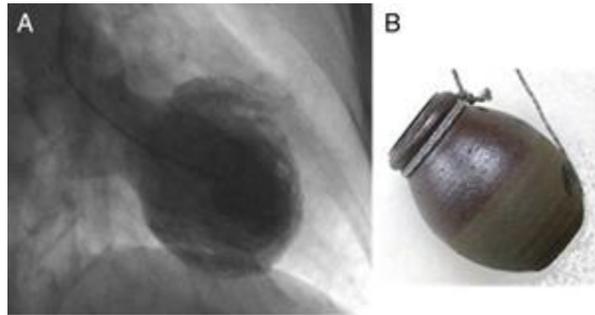

Figure 1. X-ray image of a heart showing typical apical ballooning during systole in a TTC patient. This characteristic shape resembles the shape of clay pots used in Japan to trap octopus. The first reported case was in Japan and the investigators named this peculiar condition "Tako-Tsubo", which is Japanese for octopus trap. Reprinted with permission from the *Portuguese Journal of Cardiology* [8], Copyright Elsevier (2012).

The common feature of both physical and psychological stress responses in these contexts is elevated plasma catecholamines [1, 22-24]. Indeed, studies examining plasma catecholamine concentrations in TTC patients have shown high levels of circulating catecholamines compared to those from control groups [22, 25]. The fact that exogenously administered catecholamines can induce TTC-like symptoms in patients and animal models further supports adrenergic mediation of this syndrome [16, 26-28]. As mentioned above, TTC symptoms have also been reported in some patients with pheochromocytoma, which results in abnormally high concentrations of circulating endogenous catecholamines secreted from adrenal medullary tumors [29]. TTC symptoms have responded favorably to pharmacological intervention with beta-blockers in some cases [12]. Taken together, these observations strongly implicate the major peripheral catecholamines, adrenaline and noradrenaline, as key players in acute precipitation of TTC.

The central objective of this review is to critically evaluate three distinct hypotheses put forward to describe the pathophysiology and mechanism of the clinical phenotypes associated with TTC. For the sake of simplicity, we have grouped the three main hypotheses into general categories that will be referred to as (i) *Vascular Microspams* [6, 22], (ii) *Differential β-adrenergic receptor distribution* [2, 10], and (iii) *Differential Regional Adrenergic Stimulation* [1, 3]. We define and discuss each of these hypotheses in separate sections that follow, but first briefly describe the main clinical characteristics, treatment and history of TTC.

Clinical Presentation

TTC is commonly misdiagnosed as Acute Coronary Syndrome (ACS) or ST-segment elevation myocardial infarction (STEMI) due to similarities in their clinical presentation [30, 31]. As a result of growing awareness of this issue, the United States and international guidelines include TTC as a differential diagnosis for ACS [32]. Ischemic chest pain and dyspnea are the two most common presenting symptoms of TTC [33]. Other symptoms reported include palpitations, syncope, shock, respiratory arrest, abdominal pain, myalgia, aphasia, ataxia, and sudden death [33-36].

One of the main clinical features of TTC is akinesis or dyskinesis of the LV. Originally, akinesis of the apex was described as causing an "apical-ballooning" phenomena during systole. The apical wall motion abnormality is considered the "typical" TTC variant but other morphologies have been described (Figure 2) [4]. In the mid-ventricular variant, for example, the areas

around the mid-ventricles do not contract adequately during systole. A reverse TTC variant affects the base of heart instead of the apex [4]. During systole, the basal akinesis or hypokinesis cause an apparent "ballooning" of the myocardium at the base because the rest of the ventricle contracts as usual, but the affected areas do not and, hence, remain in relaxed condition during contraction, which gives the appearance of ballooning during systole when viewed using in vivo imaging techniques such as angiography, echocardiography, and magnetic resonance imaging (MRI). Other localized variants affect limited regions of the LV and cannot be categorized into one of the three previously described morphologies [4]. These areas of wall motion abnormalities are not within the distribution of a single coronary artery [12].
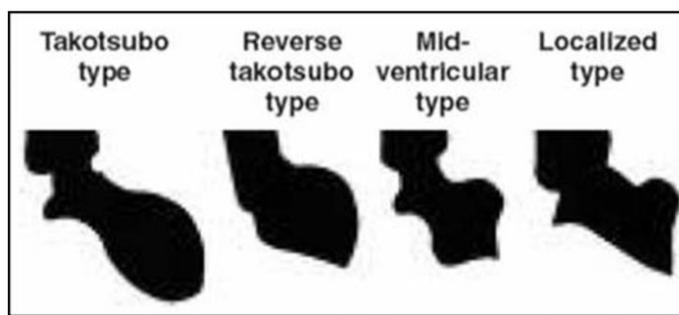

Figure 2. Different forms of TTC. The most commonly reported form is the Takotsubo type. Reprinted with permission from *Journal of Cardiology* [4]. Copyright, Elsevier (2006).

Many TTC patients present with a low left ventricular ejection fraction [37]. One review of case studies found that mean ejection fraction in TTC patients ranged from 20-49% compared with the normal range of 60-76% upon follow-up [38]. EKG changes were reported in a many cases. ST-segment elevation was the most common EKG abnormality followed by T-wave inversion [33, 39]. Other changes seen less frequently are ST-segment depression, peaked T-wave, flattened T-waves, QT-prolongation, and Q-waves [6, 12, 36, 39]. These changes are transient and usually resolve within a few months. Cardiac enzymes, such as troponins, creatine kinase, and brain natriuretic peptide (BNP), are usually elevated [33, 39]. One review of case studies found 86% of patients had elevated troponins [38].

Another literature review found only 10.7% of patients had normal or absent enzymes [39]. EKG changes in TTC cannot be readily distinguished from EKG changes found during a myocardial infarction (MI) in the absence

of coronary angiography. In some cases, however, cardiac enzymes in TTC can be differentiated from MI by the amount detected with milder elevations in enzymes seen in TTC compared to an MI [39].

Another distinction of TTC is a preceding stress-related event, though this may not be the sole distinguishing feature since stress has also been reported to trigger MI and other cardiovascular events [40].

Physical and emotional stress such as death of a loved one, public speaking, motor vehicle accidents, arguments, natural disasters, alcohol, and surprise parties have been reported before the patient experiences symptoms of TTC [12, 13, 41]. TTC patients have a higher prevalence of anxiety disorders, which may predispose them to developing this disease [36]. Medical procedures, acute medical illnesses, and opiate withdrawal can also trigger the cardiomyopathy [12, 35].

Specific pathophysiological mechanisms leading to TTC are still a matter of debate, though there has been a suggestion of an underlying genetic component since only a subset of postmenopausal women are susceptible and because familial cases have been reported [42, 43]. The American Heart Association, however, has classified TTC as a primary acquired cardiomyopathy [44], which deemphasizes the genetic component of this disease.

Formal diagnostic criteria for TTC have been proposed [36]. Of these, the Mayo Clinic diagnostic criteria appear to be the most widely accepted [45]. These criteria are defined as follows:

1. "Transient hypokinesis, akinesis, or dyskinesis of the left ventricular mid segments with or without apical involvement. The regional wall-motion abnormalities extend beyond a single epicardial vascular distribution.
2. Absence of obstructive coronary disease or angiographic evidence of acute plaque rupture.
3. New ECG abnormalities (either ST-segment elevation and/or T –wave inversion) or elevated cardiac troponin.
4. Absence of:
   • Recent significant head trauma
   • Intracranial bleeding
   • Pheochromocytoma
   • Myocarditis
   • Hypertrophic cardiomyopathy"

Diagnostic Evaluation

The "gold-standard" for diagnosing TTC is coronary angiography and left ventriculography [46]. Cardiac catheterization and angiography is helpful in determining ejection fraction and stenosis of vessels. As the criteria proposed by Prasad states, TTC patients typically do not have coronary artery disease, or if there is mild to moderate atherosclerosis it cannot account for the wall motion abnormalities [12, 45, 47]. If severe stenosis is present and there is evidence of coronary artery disease (CAD), acute coronary syndrome will more likely be an appropriate diagnosis. Cardiac catheterization has also found vessel spasms and aberrant coronary microcirculatory function at the time of presentation. The significance of the spasm and abnormal circulation has not been determined [12, 39]. Left ventriculography allows visualization of wall motion abnormalities [35, 38, 48]. EKG and cardiac enzymes levels should be obtained in patients describing ischemic chest pain to rule out major cardiac conditions such as ACS, STEMI, and arrhythmias. Since one of the diagnostic criterion for TTC is transient changes in EKG and cardiac enzymes, it is also important to perform these tests to establish a baseline and observe any changes for diagnosis [12].

In an emergency setting, echocardiograms are the preferred imaging modality. Echocardiograms are non-invasive and can assess potential complications. Similar to the left ventriculograph, it shows apical ballooning or other dyskinesis of the ventricular walls [38, 46, 48]. Cardiac magnetic resonance imaging (CMRI) with gadolinium contrast can help to discriminate between ACS, myocarditis, and TTC in obscure cases, such as those with elevated cardiac enzymes and EKG changes [46]. In TTC, CMRI will not show contrast enhancement whereas ACS and myocarditis will, due to myocardial edema [49]. CMRI is also useful in detecting a thrombus missed on echocardiogram [50].

Complications and Prognosis

One retrospective data analysis looked at the clinical course of 107 TTC patients and analyzed their cardiac complications [51]. Cardiac complications found in these patients included left ventricular outflow obstruction, mitral regurgitation, pericardial effusion, coronary artery stenosis (that did not account for the abnormal wall motion abnormalities), atrial fibrillation, atrial flutter, cardiac death, pump failure, ventricular tachycardia, ventricular

fibrillation, and atrioventricular (AV) block [51]. Apical thrombus is another cardiac complication that has been reported in a different study. Thrombi form in 2.5-9% and thromboemboli form in 0.8-14% of cases and are thought to be due to hypokinesis or stasis of the left ventricle. Thromboembolism can also lead to stroke and neurological deficits [36]. Cardiogenic shock and thromboembolism are the most common causes of death in these cases [33]. Rupture of the septum, free wall, and papillary muscles can also occur [12, 36]. In one-third of patients right ventricle dysfunction as well as LV is observed [12]. These patients have a worse prognosis with more severe heart failure, longer hospital stays, and greater hemodynamic instability [12]. Non-cardiac complications include pulmonary edema, found in 0-44% of cases, and pneumothorax, which is rare [33, 52].

TTC is transient and has a relatively good outcome with low in-hospital mortality. Recovery of left ventricular function and ejection fraction occurs over days to months. Mean ejection fraction on follow-up ranged from 60-76% [13, 38]. Prognosis appears to be dependent on ventricular function [36]. T-wave inversion, elevated white blood cells (WBC), BNP, and c-reactive protein (CRP) has been associated with poor outcome [36, 51]. Recurrence rate is 2-10% within the first few years.

Treatment

There has yet to be a clinical trial for treatment of TTC; therefore, no standardized treatment protocol is currently in place. Current practice is thus highly variable, but appears to mostly be aimed at supportive and preventative care. For example, in patients that were hemodynamically unstable, intra-aortic balloon counterpulsation, fluids, beta-blockers or vasopressors have been used [53]. Anticoagulation is recommended to reduce risk of thrombus formation and embolization. Echocardiograms should be performed if heart failure is worsening, before discharge, and on follow-up if left ventricular function had not normalized at discharge [12, 36].

Epidemiology and Prevalence

A disproportionate number of patients affected by TTC are postmenopausal women [12, 52, 54]. According to one review, 82-100% of patients affected by TTC were women [12]. The average age of these patients

ranged from 61-76 years, and only a small percentage (2.7-3%) were under the age of 50 [36, 38, 52]. Reported cases, however, ranged from 10 to 91 years old [12, 38]. In cases that reported race, the majority of patients diagnosed were Asian women followed by Caucasian women [33]. Caucasians more commonly had T-wave inversion on EKG, were younger than their Asian counterparts, presented with chest pain, and were more sensitive to emotional stress. While Asians tended to have ST-elevations, were older (70.4 years old), less likely to have preceding stress, and had greater mortality rate [33]. Although early cases of TTC were reported predominantly in Japanese literature, cases have been reported worldwide [12, 33]. It is estimated that approximately two percent of patients diagnosed with ACS may actually be patients with TTC cardiomyopathy [55]. One study following intensive care unit patients admitted for non-cardiac diagnosis found 26 of 92 patients had decreased ejection fraction, and increased incidence of apical ballooning [56].

Role of Sex (Gender), Age, and Hormonal Status

As mentioned above, TTC is predominately found in female patients who have undergone menopause [33]. In conglomerate, it has been estimated that approximately 90% of documented TTC cases were postmenopausal women [12, 37, 48, 50, 57-60]. Perhaps not surprisingly, this has led researchers to suspect a connection between female hormone status and TTC susceptibility [61-65]. Ovarian failure, when follicles in the ovaries have been eradicated and the female can no longer enter ovulation, is a characteristic sign of menopause. Due to the lack of follicles the female can no longer produce estrogen and progesterone [66].

Studies in animal models have suggested that estrogen may provide some protection from TTC [67, 68]. For example, estrogen supplementation to ovariectomized rats has been shown to attenuate some of the symptoms associated with TTC [63-65, 67, 68]. So far, all of these studies have been conducted in rats by the Ueyama group. Some primate studies have been done to examine TTC, however, these did not evaluate the role of gender or female sex hormones (only male cynomoglus monkeys were used) [28]. Thus, there is a need for additional animal model studies to help elucidate mechanisms. Primates are expensive and there are many ethical concerns about their use for stress research. Alternative large animal models such as pigs, dogs, or sheep may prove useful, but to the best of our knowledge, these have not yet been developed for TTC. Mid-sized animal models like the rabbit have proved

useful for examining gender-related cardiovascular differences [69-73]. Mice may also serve as an attractive complement to rats as a TTC model since genetic components can be relatively easily manipulated in this model. Several studies have shown that estrogen has significant influence over cardiac ion channels and function in this model [74-77]. Although the effects of gender and estrogen have not yet been carefully examined in the mouse model, Shao et al. [27] have recently described TTC-like induction in mice following administration of a single high dose of the beta-agonist, isoproterenol, and have made the novel observation that lipotoxicity may be a contributing factor, though the doses of isoproterenol required to induce TTC were extraordinarily high. This caveat notwithstanding, there has been a reported association between hyperlipidemia as a risk factor for TTC in human patients [78], and the observed lipotoxicity in this mouse model may aid our understanding of how dysregulation of lipid metabolism may affect TTC outcomes. Further development of these and related animal models should ultimately prove useful for elucidating the underlying mechanisms leading to the onset and severity of TTC.

In contrast to the paucity of animal model data, there have been many retrospective clinical studies on TTC and gender differences [79]. According to one study, 93.5% of the patients diagnosed with TTC were female [33], though the authors noted that once a TTC attack has taken place, the clinical outcome appeared similar regardless of gender [33]. Male and female patients displayed similar complications during TTC [79]. The differences concerning TTC seem to take place before an attack occurs, and less so afterwards [80], though this has not been uniformly established due to lack of carefully controlled studies. Retrospective studies also suffer from relatively few male patients for comparison. Nevertheless, the mortality rates measured between genders showed no significant differences despite the fact there were certainly individual differences from both gender categories [80]. Clearly, additional research is needed to determine the true impact and consequences of TTC in males relative to females.

A study done on TTC compared differences between male and female patients [21]. There were no differences in the psychiatric health of the patients for this study. The psychiatric health parameters reviewed included anxiety, migraines, and depression. Despite this, males and females appeared to have different triggers for TTC. Male patients with TTC previously had some type of physical stress stimulus (surgery, embolism, or pancreatitis) [21]. On the other hand, female TTC patients usually experienced a specific emotional event (death of relative, divorce, or notice of debts) in their lives [33]. Another

interesting note made through the study was the difference between treatment regime when comparing males and females. Males presented with lower ejection fractions than females affected by TTC. This influenced the reason(s) males had an increase in mechanical ventilation (replaces or assistance in natural respiration) as a treatment option during the attack. A review of 224 TTC patients in the United States indicated that male patients were able to endure longer amounts of time with increased catecholamines before developing TTC, while female patients developed TTC much more quickly brought on by an apparent surge of circulating catecholamines [21].

An anatomical difference between males and females relating to TTC is the size of the LV [33]. Males have a larger LV than females on average. This size difference could be one reason that females are more affected by TTC than males. When catecholamines increase in circulation the female LV is more prone to an outflow tract obstruction than the male LV. This could cause the base of the LV to hypercontract resulting in left ventricular outflow tract (LVOT) obstruction. It remains to be determined how prevalent these issues are in males and females since LVOT obstructions have been documented in only a very small number of TTC cases to date [33].

To summarize this section, there is strong clinical evidence showing the vast majority of TTC cases are postmenopausal women. Men and premenopausal women tend to have different precipitating factors compared to postmenopausal women who typically had specific emotional stressors that triggered the TTC episode(s). Hormonal status is a likely risk factor for TTC, and some studies have shown correlations between declining female sex steroid hormone levels and TTC incidence. This hypothesis is supported from animal model (rat) data showing that estrogen can attenuate TTC. Despite much progress and attention in recent years, there remain many mysteries regarding the roles of biological sex and sex steroid hormones with respect to TTC. Some of the key unanswered questions include the following:

1. Does the loss of estrogen and/or progesterone truly lead to increased TTC susceptibility?
2. Conversely, can estrogen and/or progesterone provide protection from TTC?
3. Does testosterone provide protection from TTC?
4. How do sex steroid hormones influence TTC susceptibility? (i.e., what are the targets?)
5. Are TTC "triggers" truly more emotionally-induced in postmenopausal women compared to men or premenopausal women?

6. Are age and sex (gender) independent risk factors (e.g., from menopausal status) for TTC?

Answers to these important questions will require additional research. New and improved preclinical animal models of TTC would also help to address these issues. Most clinical studies focused on TTC to date have all been retrospective, which is a current limitation of our knowledge in this area. In silico models hold promise for testing TTC mechanistic hypotheses, but these work best in conjunction with patient data and/or relevant experimental data from animal models. Aside from the issues of risk factors as they relate to sex, menopausal status, and emotional triggers, there are even more compelling unanswered questions regarding the acute pathophysiological mechanisms responsible for the peculiar cardiovascular effects seen in TTC patients. In the following section, we critically examine three distinct competing hypotheses that have been put forward to explain the biological basis for TTC.

Hypothesis I: Vascular Microspasms

The pathogenesis of TTC has been debated since the description of this unique cardiovascular condition. One early hypothesis has become controversial since it was first proposed by Sato et al., 1990 [7] stating microvasculature dysfunction is the precipitating factor that causes TTC [6]. In this study they showed spontaneous multi-vessel spasms in two patients, and another two showed similar symptoms following an ergonovine provocation test [6]. Ergonovine, as well as acetylcholine (ACh) provocation tests, are commonly performed to diagnose patients with coronary artery spasms that present without occlusion of coronary arteries [81]. Briefly, the tests are performed by administering graded doses (25-100 μg) of the drug into the left coronary artery and then the right coronary artery. If no chest pain, EKG change, or coronary spasm is observed, the dose is increased until the maximum dose (100 μg) is administered, followed by infusion with nitroglycerin to ameliorate symptoms. Coronary spasm is typically defined by reduction of the epicardial arteries by greater than 75% (Figure 3) [82].

After the initial description of coronary spasms in the affected areas of TTC multiple case studies were published supporting this hypothesis. One such study produced vascular spasms in 10 of 14 patients, with four patients experiencing single epicardial coronary spasms and six experiencing multivessel coronary spasms [60]. Using myocardial contrast echocardiography, 11 of 14 patients were assessed for and diagnosed with a

myocardial perfusion defect within the left ventricular apical myocardium that was ameliorated by the infusion of adenosine [83].
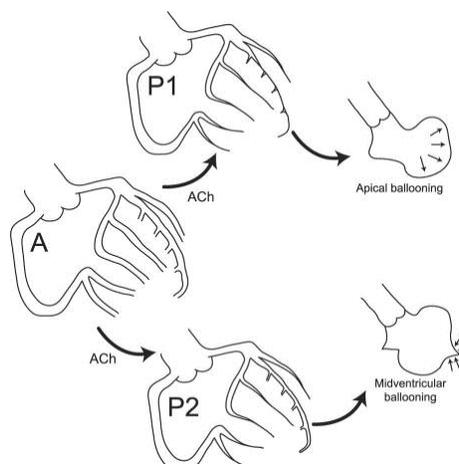

Figure 3. Cartoon depiction of Ach-induced coronary vasospasm test showing how it may lead to apical (P1) or mid (P2) ventricular ballooning. Reprinted with permission from *Texas Heart Institute Journal* [9]. Copyright 2010 by the Texas Heart Institute, Houston..

Other methods used to evaluate coronary vascular dysfunction include Thrombolysis in Myocardial Infarction (TIMI) flow grade. This methods utilizes the number of cineframes it takes for a dye to reach a location during angiography [84]. In a case study of patients presenting with TTC at the Mayo Clinic, Rochester, MN (Jan 2002-Dec 2003) all 16 patients had larger mean TIMI frame counts in the left anterior descending, left circumflex and right coronary arteries than matched controls (p less 0.001 for each) [85]. Similarly, a study including 28 patients showed significantly higher TIMI frame counts in all three arteries when evaluated with a coronary angiogram [59]. Moreover, assessment of the TIMI myocardial perfusion grade (TMPG), dysregulation of perfusion was measured in 29 of 42 patients (69%) in a retrospective study conducted at Mayo Clinic, Rochester, MN [86].

Using an alternative method to evaluate coronary microvascular function, coronary flow reserve (CFR) quantification was obtained during transthoracic doppler echocardiography in 20 consecutive patients [87]. It was found that CFR increased by a mean of 40% in patients between the acute phase of TTC and recovery phase [87]. Not unlike CFR, coronary flow velocity reserve (CFVR) was measured using a doppler guidewire in patients in the acute phase

of TTC and at follow-up. In all patients (n=8) CFVR was increased in the three coronary arteries at follow-up appointments as compared to initial presentation [88].

Although these early studies proposed coronary microvascular spasms as an underlying pathophysiological mechanism as the cause for TTC, many subsequent reports have failed to provide substantial supporting evidence for this hypothesis. One example of this was observed when only three of 212 (1.4%) patients examined demonstrated spontaneous multivessel epicardial spasms, and a mere 24 of 84 (28.6%) demonstrated multivessel epicardial spasms even after a provocation test (ergonovine and/or acetylcholine) [38]. Abe *et al* evaluated coronary microvascular spasms using acetylcholine provocation testing, however, of the seven patients evaluated only one patient showed coronary vasospasm and four demonstrated diffuse vasoconstriction. This led the group to conclude that, "coronary vasospasm does not contribute to the etiology" of TTC [89]. Others have repeated provocation tests with similar results, demonstrating only two of seven patients experienced coronary spasms and a more drastic study showed zero of 47 cases displayed vasospasms in the epicardial coronary arteries [80, 90].

These contradicting reports have dampened early enthusiasm supporting the hypothesis of microvascular coronary spasms as the pathogenesis of the observed apical ballooning. While this hypothesis was the prominent etiology most favored initially, it has declined in popularity due to discrepancies between studies indicating that coronary vasospasms may not be the most probable mechanism for the induction of TTC. This does not mean, however, that coronary microspasms are not contributory in TTC. It is nearly impossible to continuously record and capture early triggering events in patients, and so it is also not possible to say for certain that vasospasms do not occur in most cases. Consequently, the vasospasm hypothesis remains viable despite its apparent decline in popularity. Further work is required to determine the extent to which vasospasms may or may not underlie TTC mechanisms.

Hypothesis II: Differential Regional β_2-receptor Expression and Signaling (G_s/G_i Switch)

An alternative theory to the pathogenesis of TTC proposed that increased expression and stimulation of β_2-adrenergic receptors in the left ventricular apex as compared to right ventricle and the basal portions of the heart may lead to selective myocardial stunning [10]. This hypothesis is supported, in part, by a study that examined β-receptors in the canine heart and found there was greater β-adrenergic receptor sensitivity in the apex due to significantly

greater cAMP responses to challenge with noradrenaline or a forskolin derivative (stimulates adenylate cyclase) were observed compared to basal regions. Further, β-receptor densities were found to be significantly ($p<0.05$) higher in the apical regions of the heart as compared to the base (B_{max} = 455±45 vs. 341±35, respectively, n=5) [91]. The authors speculated the increased receptor density in apical regions may be a physiological adaptation to compensate for lower sympathetic nerve input to the apex. Highest nerve terminal densities in the ventricle are concentrated at the base [92]. Similar differential apical-base responsiveness to isoproterenol was also observed in the feline, rat, and rabbit models [93-95]. These studies suggested enhanced β-adrenergic receptor expression and/or sensitivity in apical regions of the LV may be a conserved feature of mammalian cardiac physiology. These studies did not examine β-receptor subtype distribution, so the relative ratio of β_1 to β_2-adrenergic receptors in apex versus base was not resolved in these studies.

A recent study by Paur et al. [2] did measure β_2-adrenergic receptor binding in myocytes isolated from base versus apex in the rat model, and found that apical myocytes had significantly higher β_2 densities than those isolated from the base. This study also examined an adrenaline-induced shift from G_s (stimulatory G protein signaling) to G_i (inhibitory G protein signaling) in the rat heart model, and found that adrenaline, unlike noradrenaline, produced a negative inotropic response in apical and mid, but not basal regions of the LV [2].

The authors hypothesized this negative inotropic effect was the result of high-dose adrenaline stimulation of β_2-adrenergic receptors thereby causing myocardial stunning (sometimes referred to as "neurogenic stunning"), a condition whereby the myocytes cease to contract and, in fact, become refractory to further stimulation. Under these conditions, the β_2-adrenergic receptors are thought to be phosphorylated at specific residues by both protein kinase A (PKA) and G-protein receptor kinase (GRKs), resulting in a switch from G_s to G_i. This was shown by pretreatment with pertussis toxin (PTX) to prevent the G_s to G_i shift, resulting in complete ablation of the negative inotropic effects in apical and mid-regions of the LV following adrenaline injection [2, 26].

The authors suggest that circulating adrenaline, which is typically elevated in TTC patients, induces TTC through hyperactivation of β_2-adrenergic receptors preferentially in apical and sometimes mid-regions of the left ventricular wall, leading to selective myocardial stunning in these areas due to the switch from G_s to G_i coupling mechanisms, as originally postulated in a 2008 review by Lyon et al. [10].

This hypothesis helps to explain how stress-induced myocardial stunning may preferentially affect apical myocardium in the LV to produce TTC-like symptoms. There are, however, a number of inconsistencies and perplexities that call into question the general validity of this hypothesis. One of the most striking of these are a number of recent reports from the clinical literature showing that accidental adrenaline overdose (e.g., from EpiPen® or similar) can produce TTC-like symptoms, but these are characterized by hypokinesis of the base rather than apex, leading investigators to refer to this variation as "Reverse or Inverted TTC" [17, 96-98]. Studies have reported a higher percent of the inverted TTC patients reported were significantly younger than mid and apical cases of TTC [99, 100]. If there is truly a higher β_2-adrenergic receptor density at the apex, then why is basal hypokinesis seen in adrenaline-induced cases?

Another challenge to this hypothesis stems from case reports showing that infusion of dobutamine, a β_1-selective adrenergic agonist, led to TTC as seen in Figure 4 [5, 16]. According to the G_s/G_i theory, β_1-adrenergic stimulation does not induce the switch to G_i, and therefore should not lead to myocardial stunning.

The results from the rat model showed that β_1-selective adrenergic receptor stimulation did not induce TTC-like symptoms [2]. This may be a limitation of the model system, but clearly there are clinical situations where human patients develop TTC-like symptoms with dobutamine. Interestingly, there was some evidence of coronary vasospasms in the presence of dobutamine [5, 101], suggesting it may be working through a different type of mechanism, perhaps more akin to that described in the first hypothesis discussed. This raises the question that there may be more than one mechanism that can produce TTC.

Another study showed that metoprolol, a β_1-selective adrenergic receptor antagonist, effectively attenuated symptoms associated with adrenaline-induced TTC in cynomolgus monkeys [28]. Others have shown the increase in heart rate after isoproterenol infusion occurs in primarily a β_1-adrenergic receptor-dependent fashion [102]. In addition, cardiac β_1-receptors were shown to be increased in ovariectomized (post-menopausal) rats [103]. As stated previously, TTC affects primarily post-menopausal women, and upregulation of β_1-adrenergic receptors in the heart may contribute to this susceptibility, but it is not yet known if this happens in human patients. Nevertheless, when considered in sum, clinical evidence certainly suggests that β_1-adrenergic receptors likely play a significant role in the development of TTC.

Consequently, the question of which adrenergic receptor subtype is the key mediator for TTC remains open. Relatively few studies have even reported adrenergic receptor expression or binding data along the apex-base axis, and to the best of our knowledge, none of these studies have examined this in the human heart. As mentioned earlier, there was one classic study done in a relatively small number (five) of canine hearts that showed significant increases in β-adrenergic receptor densities at the apex versus base of the LV [91].
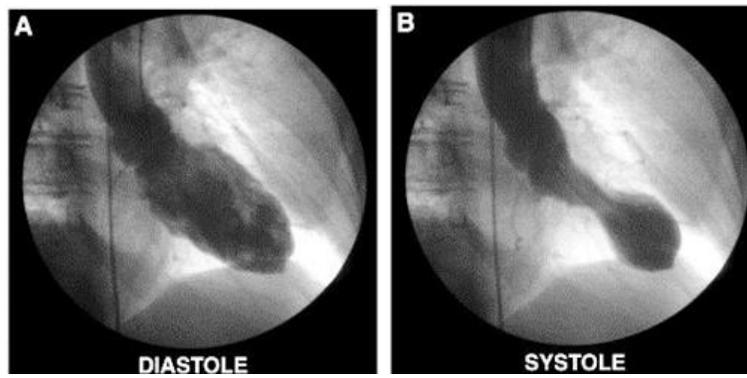

Figure 4. Case of dobutamine-induced TTC. Reprinted with permission from *International Journal of Cardiology* [5]. Copyright Elsevier (2006).

This study did not examine β-receptor subtypes. At present, only one study has shown higher densities of β_2-adrenergic receptors in apical myocytes compared to those from the base, and this was done in the rat model using isolated myocytes rather than in situ receptor binding [2]. Thus, there is a scarcity of hard data on the anatomical distribution of β_1- versus β_2-adrenergic receptor distributions along the apical-base axis of the LV, though it is well-established that both receptor subtypes are present and functional in the heart, with β_1-adrenergic receptors accounting for the majority (~67%) of these [104]. Key questions will be their relative anatomical densities, sensitivities, and functions, and how these may differ depending on age, sex, and/or hormonal status.

Another consideration that should not be overlooked is the involvement of α-adrenergic receptors. The blockade of the α_1-receptor, in combination with β_1-receptor blockers, was shown to diminish the effects on immobilization stress (IMO)-induced cardiomyopathy in a rat model [61, 67, 105]. Further, a calcium channel blocker, azelnidipine, has been reported to prevent TTC in a

rat IMO model [106]. Although these are isolated reports, they nevertheless demonstrate that TTC is complex and there may be multiple mechanisms involved.

Hypothesis III: Differential Regional Cardiac Catecholamine Release/Overload

A third distinct hypothesis that has been put forward to explain TTC is that local release of catecholamines could overload adrenergic receptor signaling systems in specific regions of the heart [1, 3]. During stress, there is input from sympathetic nerves as well as circulating catecholamines, but there are also autocrine/paracrine actions of catecholamines from stores within the heart itself [3, 107-113]. As mentioned earlier, sympathetic nerve input is not uniform throughout the LV. There is much greater nerve terminal density in the base compared to mid or apical sections of the LV [114, 115]. Kume et al. [1] measured catecholamines from two locations representing base (aortic root, Ao) and apex (coronary sinus, CS) in five confirmed cases of TTC. In all five cases, concentrations of noradrenaline and dopamine were elevated in CS compared to Ao, whereas adrenaline concentrations were unchanged or slightly decreased (Figure 5) [1]. These results suggested there may indeed be differential local catecholamine concentrations in apical and basal regions of the left ventricular myocardium.
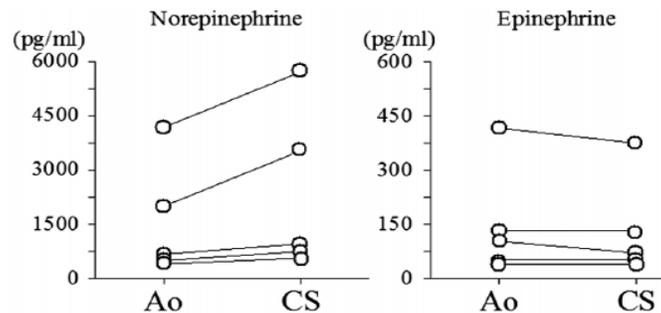

Figure 5. Catecholamine concentrations measured in 5 TTC patients from regions in the base (aortic root, Ao) or apex (coronary sinus, CS). Reprinted with permission from *Circulation Journal* [1].

Another piece of evidence comes from recent studies in the mouse heart showing cells marked by expression of the adrenaline biosynthetic enzyme,

phenylethanolamine n-methyltransferase (Pnmt), were found to be concentrated on the left side of the adult heart [3]. More specifically, Pnmt+ cells were localized to swaths or finger-like projections into left ventricular myocardium at the apex, mid, and basal regions as shown in Figure 6 [3]. These data suggest there may be an anatomical substrate for regional catecholamine production differences in the heart. Nearly 90% of Pnmt-derived cells were found to be localized to the left side of the heart, which could, in theory, help to explain why left ventricular function is selectively influenced in TTC. Moreover, the regional variations within the LV could likewise help to explain how those areas could be susceptible to local surges in catecholamines. It is also possible that local presence of catecholamines in these regions during development may influence the expression and functional sensitivities of adrenergic receptor subtype distributions in the LV. Although this has not been explicitly demonstrated in the heart, there are numerous studies showing that innervation and adrenergic receptor expression is influenced by catecholamine exposure [116-118].
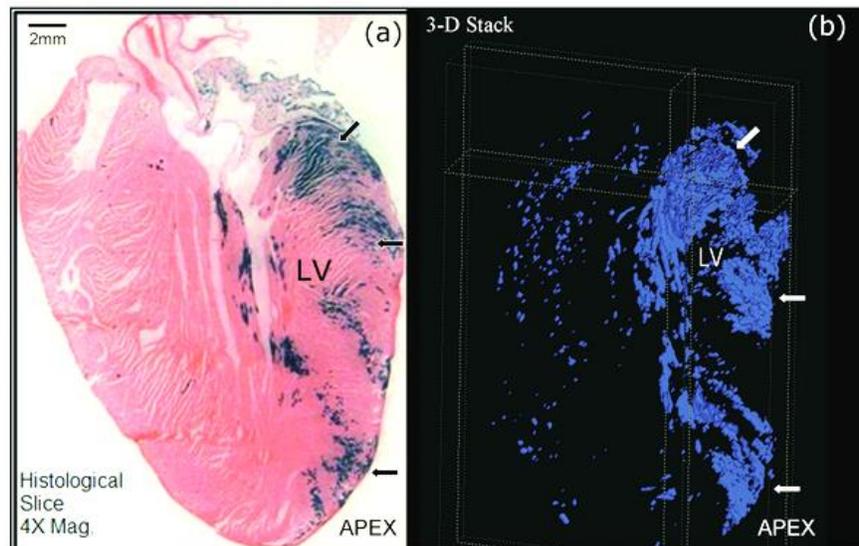

Figure 6. (a**)** Adrenergic (Pnmt+) myocardium is stained blue with XGAL in the mouse heart. (b) Three-dimensional reconstruction of adrenergic cell staining throughout the LV. Note the concentrations of blue finger-like projections into the apex, mid, and base regions of the LV (arrows). Reprinted with permission from *Plos One* [3].
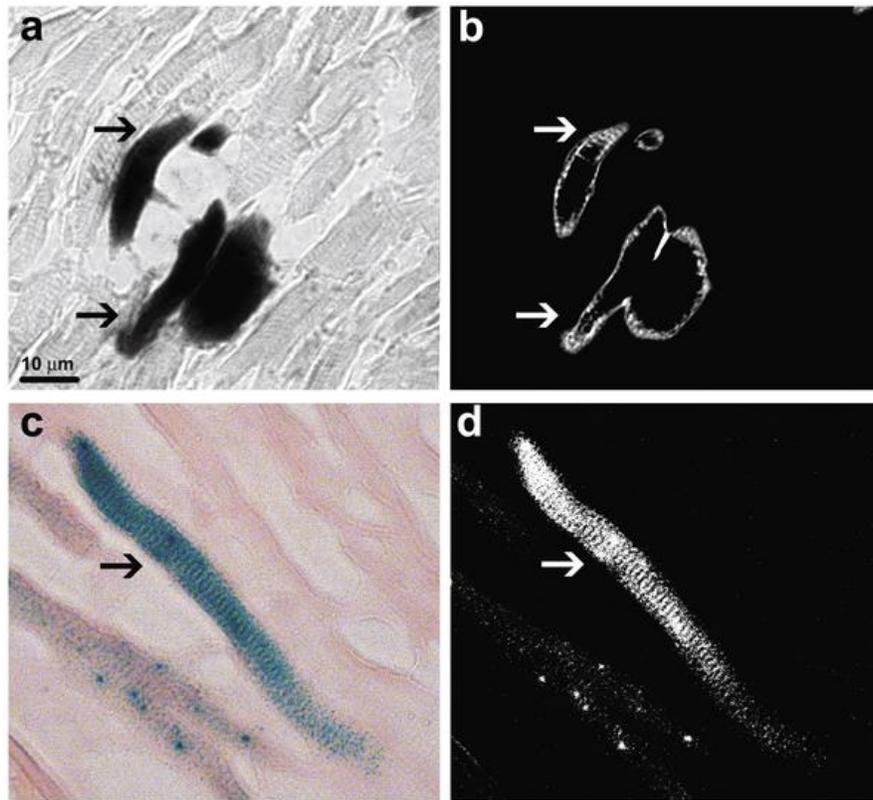
Figure 7. Pnmt-marked (XGAL) adrenergic cells in adult mouse LV have myocyte characteristics as shown by from the ladder-like striations clearly visible in XGAL-stained cells from these hearts (arrows). Left panels show phase-contrast images; Right panels show inverted phase-contrast images of the corresponding cells shown on left. Reprinted with permission from *Plos One* [3].

Notably, many of the Pnmt-derived cells in the adult mouse heart appear to be myocytes, though neuronal-like and immature embryonic-like Pnmt-derived cells were also identified in these regions [3]. Clear examples of striated myocardial cells marked with XGAL to denote Pnmt expression were observed in the LV (Figure 7) [3, 119]. These results suggest there may be autocrine or paracrine actions of local catecholamines from non-neuronal as well as neuronal sources. This idea is not new [120-122], but is often overlooked in modern studies and textbooks. The classic work of Spurgeon et al. [123] showed that cardiac adrenaline concentrations remain relatively high in the heart following surgical and chemical (6-hydroxydopamine, 6-OHDA)

denervation, while noradrenaline concentrations were nearly completely eradicated by these procedures. The authors concluded that cardiac noradrenaline content was mostly neuronal while only ~50% of the adrenaline content was neuronal. They hypothesized that cardiac adrenaline "is held in non-neuronal stores either in chromaffin cells, in the specialized cells themselves or in cardiac analogs of chromaffin cells" [123]. There is ample evidence for these from a wide variety of species [124-127], including humans [128], suggesting their presence is highly conserved and, therefore, likely critical for survival. It remains to be determined if these cells truly play a significant role in TTC, but they certainly represent a potential local source of adrenaline that could contribute to regional variations in adrenergic stimulation within the LV.

There are still many unanswered questions about this hypothesis. We do not know, for example, if there are changes in cardiac adrenaline content or regional differences in the distribution or concentrations of local catecholamine production in the heart in response to aging, sex, or hormonal status. One conundrum with this hypothesis is the heaviest concentrations of Pnmt-marked adrenergic cells are located at the base of the LV, yet the apical and mid sections of the LV are much more frequently affected in clinical cases of TTC. The base concentrations of adrenergic cells could conceivably contribute to inverted forms of TTC, but it is still a mystery why sometimes base and other times the mid or apex are most affected. Clearly, more work is required to elucidate these mechanisms.

In addition, no-one has yet demonstrated that non-neuronal sources of cardiac catecholamines are secreted during emotional or physical stress. It is still unclear how these cells are regulated, and how they influence their local environment, though there are several reports in rodent models showing that cardiac Pnmt expression is upregulated by glucocorticoid hormones, which themselves are typically elevated in the circulation during stress responses [129, 130]. Similar concerns exist for stimulation of sympathetic nerves and the intrinsic cardiac nervous system, which in the human heart is estimated to contain at least 14,000 neurons [113]. Further research is required to delineate how local sources of catecholamines from autocrine/paracrine or neuronal inputs may impact regional myocardial function at baseline and under different types of stressful conditions.
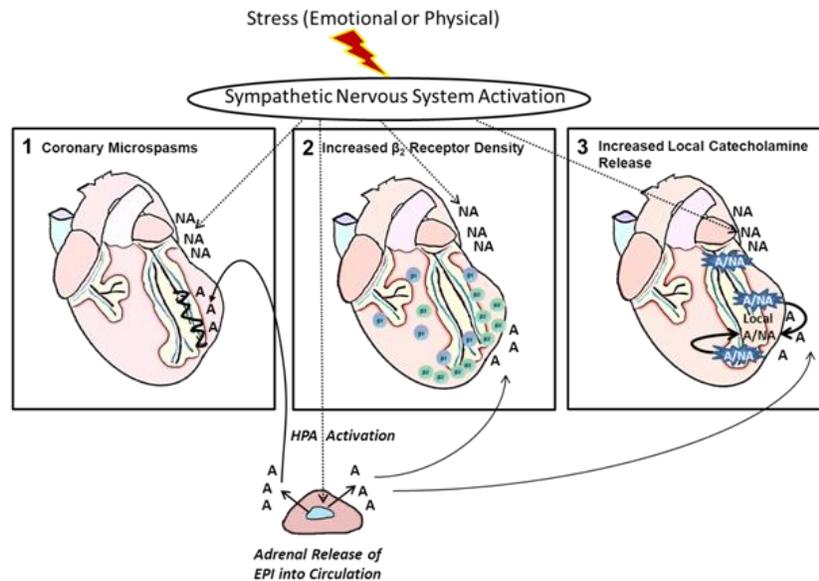
Figure 8. Schematic illustrations of three distinctive models proposed to explain TTC mechanisms. 1. Coronary Microspasms: In this model, stress-induced surges in catecholamines trigger coronary microspasms, which then lead to decreased blood flow and regional myocardial inactivity. 2. Increased β_2-Receptor Density: This model proposes that β_2-adrenergic receptors are more concentrated at the apex compared to the base. High concentrations of adrenaline cause these receptors to switch from G_s to G_i, which results in myocardial stunning in affected regions that would appear as akinetic or hypokinetic sections of LV during systole. 3. Increased Local Catecholamine Release: In this hypothesis, adrenaline and/or noradrenaline are released locally in the apex, mid, and/or basal regions of the LV from non-neuronal resident adrenergic cell populations (depicted by the highlighted blue areas). This local release overstimulates adrenergic receptors that are already near saturation from circulating adrenaline and sympathetic nerves (NA). Abbreviations: HPA, Hypothalamic-Pituitary-Adrenal (axis); A, adrenaline; NA, noradrenaline.

Summary, Conclusion and Future Directions

TTC is a complex clinical condition characterized by left ventricular regional akinesis/hypokinesis resulting in severe chest pain and ST-elevation on EKG. Episodes are typically precipitated by acute emotional or physical stress, and the vast majority of TTC cases have been reported in

postmenopausal women. There is strong evidence implicating elevated catecholamine levels as a critical factor in TTC induction, but it is not yet clear if they are coming from the circulation (adrenal secretion), sympathetic innervation, and/or local autocrine/paracrine sources. The relative inputs from these different sources may vary from individual to individual, and will likely differ depending on the type of stress impacting the individual patient. Hence, one could imagine different sorts of "catecholamine storms" [131] that could trigger different types of TTC.

Each of the three major hypotheses discussed in this chapter offer credible explanations for how adrenergic hormones may influence the development of TTC. For comparative purposes, they are each illustrated in cartoon form in Figure 8.

Although all of these hypotheses provide reasonable explanations for some aspects of TTC, none of them provide a full explanation of the underlying mechanisms leading to TTC. Each is distinctive in its features, yet they are not mutually exclusive. For example, regional variations in β_2-adrenergic receptor concentrations and/or sensitivities could result in local fluctuations inducing vasospasms in nearby coronary vessels. These could also be influenced by local release of catecholamines either from sympathetic nerve terminals or non-neuronal autocrine/paracrine sources that could result in regional overstimulation of adrenergic receptors leading to myocardial stunning, perhaps as a result of a switch from G_s to G_i coupling primarily at β_2-adrenergic receptors. Further study is required determine how these different mechanistic components of the cardiac stress response system interact to in the context of TTC.

After nearly 25 years of research since the first formal description of TTC in the published literature, we still have only a rudimentary picture of how this peculiar stress cardiomyopathy materializes. Although it was "discovered" only relatively recently, TTC has existed as an undiagnosed or misdiagnosed clinical condition for countless years. Because of the relative "newness" of TTC, many patients and some physicians are still largely unaware of the conditions and potential severity of stress cardiomyopathies, yet TTC continues to represent a real and persistent danger to susceptible individuals. In many and perhaps even most cases, TTC can often resolve on its own without interventional therapy within a matter of a few weeks [48]. This is not to say, however, that dangerous complications do not arise. On the contrary, TTC patients with manifest coronary vasospasms are increased risk for heart attacks and strokes from thrombolytic embolisms [36]. Other studies have demonstrated significant lengthening of the Q-T interval on EKGs from TTC

patients [132]. Long QT intervals are known risk factor for dangerous and life-threatening forms of ventricular arrhythmias known as Torsade de Pointes [133-135]. Several other serious complications from TTC have been noted in the medical and scientific literature, so it is by no means a benign disease [136, 137]. Continued research and critical evaluation of the results and ideas emanating from these exercises will surely help to refine our understanding of the mechanisms underlying TTC. Some of key outstanding questions include but are not limited to the following:

1. How is emotional stress "physiologically" transmitted to the heart?
2. Do different types of stress have different impacts on cardiovascular function (i.e., does it matter if the stressor elicits selective autonomic nerve stimulation versus systemic adrenaline surges)?
3. Do local non-neuronal stores of catecholamines play a part in TTC? If so, then what regulates their activities?
4. Why are only certain regions of the LV primarily affected in TTC?
5. What controls regional variations in adrenergic receptor subtype expression patterns?

These are just a few of the general questions about TTC that remain answered. We have also highlighted a number of questions earlier in this chapter pertaining to sex, age, and hormonal status. In addition, we have discussed the merits and limitations of three independent models of TTC mechanisms. Thus, while much progress has been made in the last 25 years on this subject, there is still much work to be done before we have a full understanding of the pathophysiological mechanisms responsible for this stress-induced cardiomyopathy. The three mechanistic models reviewed here offer strong frameworks for specific hypothesis testing, and thus each should be useful for designing new experiments to address some of the many still outstanding questions associated with TTC.

References

[1]   Kume, T., et al., Local release of catecholamines from the hearts of patients with tako-tsubo-like left ventricular dysfunction. *Circ J,* 2008. 72(1): p. 106-8.

[2]   Paur, H., et al., High levels of circulating epinephrine trigger apical cardiodepression in a beta2-adrenergic receptor/Gi-dependent manner: a

new model of Takotsubo cardiomyopathy. *Circulation,* 2012. 126(6): p. 697-706.

[3]   Osuala, K., et al., Distinctive left-sided distribution of adrenergic-derived cells in the adult mouse heart. *PLoS One*, 2011. 6(7): p. e22811.

[4]   Shimizu, M., et al., [Recurrent episodes of takotsubo-like transient left ventricular ballooning occurring in different regions: a case report*]. J Cardiol*, 2006. 48(2): p. 101-7.

[5]   Kumar, A., et al., Transient left ventricular apical ballooning during dobutamine myocardial perfusion imaging. *Int J Cardiol*, 2008. 124(3): p. 378-80.

[6]   Dote, K., et al., [Myocardial stunning due to simultaneous multivessel coronary spasms: a review of 5 cases]. *J Cardiol,* 1991. 21(2): p. 203-14.

[7]   H. Sato, H.T., K. Dote, T. Uchida, M. Ishihara, Tako-tsubo-like left ventricular dysfunction due to multivessel coronary spasm, in *Clinical aspect of myocardial injury: from ischemia to heart failure*, K.H. K. Kodama, M. Hori, Editor. 1990, Kagakuhyoronsha Publishing Co.: Tokyo.

[8]   Cesario, V., Loureiro, M.J., and H. Pereira, Takotsubo cardiomyopathy in a cardiology department. *Portuguese J of Cardiol,* 2012. 31(9): p. 603-8.

[9]   Angelini, P., Takotsubo cardiomyopathy: what is behind the octopus trap? *Tex Heart Inst J,* 2010. 37(1): p. 85-7.

[10]   Lyon, A.R., et al., Stress (Takotsubo) cardiomyopathy--a novel pathophysiological hypothesis to explain catecholamine-induced acute myocardial stunning. *Nat Clin Pract Cardiovasc Med*, 2008. 5(1): p. 22-9.

[11]   Steptoe, A. and M. Kivimaki, Stress and cardiovascular disease. *Nat Rev Cardiol*, 2012. 9(6): p. 360-70.

[12]   Bybee, K.A. and A. Prasad, Stress-related cardiomyopathy syndromes. *Circulation,* 2008. 118(4): p. 397-409.

[13]   Tsuchihashi, K., et al., Transient left ventricular apical ballooning without coronary artery stenosis: a novel heart syndrome mimicking acute myocardial infarction. Angina Pectoris-Myocardial Infarction Investigations in Japan. *J Am Coll Cardiol*, 2001. 38(1): p. 11-8.

[14]   Hurst, R.T., et al., Takotsubo cardiomyopathy: a unique cardiomyopathy with variable ventricular morphology. *JACC Cardiovasc Imaging*, 2010. 3(6): p. 641-9.

[15]   Sharkey, S.W., et al., Why not just call it tako-tsubo cardiomyopathy: a discussion of nomenclature. *J Am Coll Cardiol,* 2011. 57(13): p. 1496-7.

[16] Abraham, J., et al., Stress cardiomyopathy after intravenous administration of catecholamines and beta-receptor agonists. *J Am Coll Cardiol,* 2009. 53(15): p. 1320-5.

[17] Litvinov, I.V., M.A. Kotowycz, and S. Wassmann, Iatrogenic epinephrine-induced reverse Takotsubo cardiomyopathy: direct evidence supporting the role of catecholamines in the pathophysiology of the "broken heart syndrome". *Clin Res Cardiol*, 2009. 98(7): p. 457-62.

[18] Marcovitz, P.A., et al., Pheochromocytoma presenting with Takotsubo syndrome. *J Interv Cardiol*, 2010. 23(5): p. 437-42.

[19] Naderi, N., et al., Pheochromocytoma-induced reverse tako-tsubo with rapid recovery of left ventricular function. *Cardiol J*, 2012. 19(5): p. 527-31.

[20] Ennezat, P.V., et al., Transient left ventricular basal dysfunction without coronary stenosis in acute cerebral disorders: a novel heart syndrome (inverted Takotsubo). *Echocardiography*, 2005. 22(7): p. 599-602.

[21] Patel, S.M., et al., Distinctive clinical characteristics according to age and gender in apical ballooning syndrome (takotsubo/stress cardiomyopathy): an analysis focusing on men and young women. *J Card Fail,* 2013. 19(5): p. 306-10.

[22] Wittstein, I.S., et al., Neurohumoral features of myocardial stunning due to sudden emotional stress. *N Engl J Med*, 2005. 352(6): p. 539-48.

[23] Akashi, Y.J., et al., 123I-MIBG myocardial scintigraphy in patients with "takotsubo" cardiomyopathy. *J Nucl Med,* 2004. 45(7): p. 1121-7.

[24] Ito, K., et al., Assessment of Takotsubo (ampulla) cardiomyopathy using 99mTc-tetrofosmin myocardial SPECT--comparison with acute coronary syndrome. *Ann Nucl Med*, 2003. 17(2): p. 115-22.

[25] Yoshida, T., et al., A pathophysiologic study of tako-tsubo cardiomyopathy with F-18 fluorodeoxyglucose positron emission tomography. *Eur Heart J,* 2007. 28(21): p. 2598-604.

[26] Shao, Y., et al., Novel rat model reveals important roles of beta-adrenoreceptors in stress-induced cardiomyopathy. *Int J Cardiol*, 2013.

[27] Shao, Y., et al., A mouse model reveals an important role for catecholamine-induced lipotoxicity in the pathogenesis of stress-induced cardiomyopathy. *Eur J Heart Fail*, 2013. 15(1): p. 9-22.

[28] Izumi, Y., et al., Effects of metoprolol on epinephrine-induced takotsubo-like left ventricular dysfunction in non-human primates. *Hypertens Res*, 2009. 32(5): p. 339-46.

[29] Kim, S., et al., Inverted-Takotsubo pattern cardiomyopathy secondary to pheochromocytoma: a clinical case and literature review. *Clin Cardiol*, 2010. 33(4): p. 200-5.

[30] Crea, F. and G. Liuzzo, Pathogenesis of acute coronary syndromes. *J Am Coll Cardiol*, 2013. 61(1): p. 1-11.

[31] Windecker, S., et al., Future treatment strategies in ST-segment elevation myocardial infarction. *Lancet,* 2013. 382(9892): p. 644-57.

[32] Sealove, B.A., S. Tiyyagura, and V. Fuster, Takotsubo cardiomyopathy. *J Gen Intern Med,* 2008. 23(11): p. 1904-8.

[33] Donohue, D. and M.R. Movahed, Clinical characteristics, demographics and prognosis of transient left ventricular apical ballooning syndrome. *Heart Fail Rev*, 2005. 10(4): p. 311-6.

[34] Sardar, M.R., et al., Recurrent takotsubo cardiomyopathy in the setting of transient neurological symptoms: a case report. *J Med Case Rep*, 2011. 5: p. 412.

[35] Teramachi, Y., et al., Takotsubo cardiomyopathy in a senile female patient after transcatheter coil embolization of patent arterial duct. *Geriatr Gerontol Int*, 2013. 13(4): p. 1077-9.

[36] Castillo Rivera, A.M., M. Ruiz-Bailen, and L. Rucabado Aguilar, Takotsubo cardiomyopathy--a clinical review. *Med Sci Monit*, 2011. 17(6): p. RA135-47.

[37] Eshtehardi, P., et al., Transient apical ballooning syndrome--clinical characteristics, ballooning pattern, and long-term follow-up in a Swiss population. *Int J Cardiol,* 2009. 135(3): p. 370-5.

[38] Gianni, M., et al., Apical ballooning syndrome or takotsubo cardiomyopathy: a systematic review. *Eur Heart J,* 2006. 27(13): p. 1523-9.

[39] Sanchez-Jimenez, E.F., Initial clinical presentation of Takotsubo cardiomyopathy with-a focus on electrocardiographic changes: A literature review of cases. *World J Cardiol,* 2013. 5(7): p. 228-41.

[40] Jiang, W., et al., Mental stress--induced myocardial ischemia and cardiac events. *JAMA*, 1996. 275(21): p. 1651-6.

[41] Subban, V., et al., Apical ballooning syndrome in first degree relatives. *Indian Heart J,* 2012. 64(6): p. 607-9.

[42] Kumar, G., D.R. Holmes, Jr., and A. Prasad, "Familial" apical ballooning syndrome (Takotsubo cardiomyopathy). *Int J Cardiol*, 2010. 144(3): p. 444-5.

[43] Pison, L., P. De Vusser, and W. Mullens, Apical ballooning in relatives. *Heart*, 2004. 90(12): p. e67.

[44] Maron, B.J., et al., Contemporary definitions and classification of the cardiomyopathies: an American Heart Association Scientific Statement from the Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups; and Council on Epidemiology and Prevention. *Circulation*, 2006. 113(14): p. 1807-16.

[45] Prasad, A., Apical ballooning syndrome: an important differential diagnosis of acute myocardial infarction. *Circulation,* 2007. 115(5): p. e56-9.

[46] Bossone, E., et al., Takotsubo cardiomyopathy: an integrated multi-imaging approach. *Eur Heart J Cardiovasc Imaging,* 2013.

[47] Eitel, I., et al., Differential diagnosis of suspected apical ballooning syndrome using contrast-enhanced magnetic resonance imaging. *Eur Heart J*, 2008. 29(21): p. 2651-9.

[48] Bybee, K.A., et al., Systematic review: transient left ventricular apical ballooning: a syndrome that mimics ST-segment elevation myocardial infarction. *Ann Intern Med,* 2004. 141(11): p. 858-65.

[49] Dec, G.W., Recognition of the apical ballooning syndrome in the United States. *Circulation*, 2005. 111(4): p. 388-90.

[50] Sharkey, S.W., et al., Natural history and expansive clinical profile of stress (tako-tsubo) cardiomyopathy. *J Am Coll Cardiol*, 2010. 55(4): p. 333-41.

[51] Murakami, T., et al., Characterization of predictors of in-hospital cardiac complications of takotsubo cardiomyopathy: Multi-center registry from Tokyo CCU Network. *J Cardiol,* 2013.

[52] Akashi, Y.J., et al., Takotsubo cardiomyopathy: a new form of acute, reversible heart failure. *Circulation,* 2008. 118(25): p. 2754-62.

[53] Bietry, R., A. Reyentovich, and S.D. Katz, Clinical management of takotsubo cardiomyopathy. *Heart Fail Clin*, 2013. 9(2): p. 177-86, viii.

[54] Sharma, V., et al., Stress cardiomyopathy: case series and the review of literature. *J Emerg Med,* 2013. 45(4): p. e95-8.

[55] Kurowski, V., et al., Apical and midventricular transient left ventricular dysfunction syndrome (tako-tsubo cardiomyopathy): frequency, mechanisms, and prognosis. *Chest*, 2007. 132(3): p. 809-16.

[56] Park, J.H., et al., Left ventricular apical ballooning due to severe physical stress in patients admitted to the medical ICU. *Chest*, 2005. 128(1): p. 296-302.

[57] Milinis, K. and M. Fisher, Takotsubo cardiomyopathy: pathophysiology and treatment. *Postgrad Med J,* 2012. 88(1043): p. 530-8.

[58] Nobrega, S. and D. Brito, [The "broken heart syndrome": state of the art]. *Rev Port Cardiol*, 2012. 31(9): p. 589-96.

[59] Kurisu, S. and Y. Kihara, Tako-tsubo cardiomyopathy: clinical presentation and underlying mechanism. *J Cardiol,* 2012. 60(6): p. 429-37.

[60] Kurisu, S., et al., Tako-tsubo-like left ventricular dysfunction with ST-segment elevation: a novel cardiac syndrome mimicking acute myocardial infarction. *Am Heart J,* 2002. 143(3): p. 448-55.

[61] Ueyama, T., Emotional stress-induced Tako-tsubo cardiomyopathy: animal model and molecular mechanism. *Ann N Y Acad Sci,* 2004. 1018: p. 437-44.

[62] Kuo, B.T., R. Choubey, and G.M. Novaro, Reduced estrogen in menopause may predispose women to takotsubo cardiomyopathy. *Gend Med*, 2010. 7(1): p. 71-7.

[63] Ueyama, T., et al., Chronic estrogen supplementation following ovariectomy improves the emotional stress-induced cardiovascular responses by indirect action on the nervous system and by direct action on the heart. *Circ J,* 2007. 71(4): p. 565-73.

[64] Ueyama, T., et al., Cardiac and vascular gene profiles in an animal model of takotsubo cardiomyopathy. *Heart Vessels*, 2011. 26(3): p. 321-37.

[65] Ueyama, T., et al., Estrogen attenuates the emotional stress-induced cardiac responses in the animal model of Tako-tsubo (Ampulla) cardiomyopathy. *J Cardiovasc Pharmacol,* 2003. 42 Suppl 1: p. S117-9.

[66] Col, N.F., et al., In the clinic. Menopause. *Ann Intern Med*, 2009. 150(7): p. ITC4-1-15; quiz ITC4-16.

[67] Ueyama, T., et al., Emotional stress induces transient left ventricular hypocontraction in the rat via activation of cardiac adrenoceptors: a possible animal model of 'tako-tsubo' cardiomyopathy. *Circ J*, 2002. 66(7): p. 712-3.

[68] Ueyama, T., et al., Catecholamines and estrogen are involved in the pathogenesis of emotional stress-induced acute heart attack. *Ann N Y Acad Sci, 2008*. 1148: p. 479-85.

[69] Katchman, A.N., et al., Comparative evaluation of HERG currents and QT intervals following challenge with suspected torsadogenic and nontorsadogenic drugs. *J Pharmacol Exp Ther,* 2006. 316(3): p. 1098-106.

[70] Liu, X.K., et al., In vivo androgen treatment shortens the QT interval and increases the densities of inward and delayed rectifier potassium currents in orchiectomized male rabbits. *Cardiovasc Res*, 2003. 57(1): p. 28-36.

[71] Liu, X.K., et al., Gender difference in the cycle length-dependent QT and potassium currents in rabbits. *J Pharmacol Exp Ther*, 1998. 285(2): p. 672-9.

[72] Wang, W.X., et al., "Conventional" antihistamines slow cardiac repolarization in isolated perfused (Langendorff) feline hearts. *J Cardiovasc Pharmacol*, 1998. 32(1): p. 123-8.

[73] Kim, J.J., et al., Bradycardia alters Ca(2+) dynamics enhancing dispersion of repolarization and arrhythmia risk. *Am J Physiol Heart Circ Physiol*, 2013. 304(6): p. H848-60.

[74] Saito, T., et al., Estrogen contributes to gender differences in mouse ventricular repolarization. *Circ Res*, 2009. 105(4): p. 343-52.

[75] Brouillette, J., et al., Functional properties of K+ currents in adult mouse ventricular myocytes. *J Physiol,* 2004. 559(Pt 3): p. 777-98.

[76] Trepanier-Boulay, V., et al., Gender-based differences in cardiac repolarization in mouse ventricle. *Circ Res*, 2001. 89(5): p. 437-44.

[77] Wu, Y. and M.E. Anderson, Reduced repolarization reserve in ventricular myocytes from female mice. *Cardiovasc Res*, 2002. 53(3): p. 763-9.

[78] Deshmukh, A., et al., Prevalence of Takotsubo cardiomyopathy in the United States. *Am Heart J,* 2012. 164(1): p. 66-71 e1.

[79] Stollberger, C. and J. Finsterer, Why does takotsubo ("broken heart syndrome") affect more females than males? *Int J Cardiol*, 2011. 147(1): p. 175-6.

[80] Song, B.G., et al., Clinical characteristics, ballooning pattern, and long-term prognosis of transient left ventricular ballooning syndrome. *Heart Lung,* 2010. 39(3): p. 188-95.

[81] Group, J.C.S.J.W., Guidelines for diagnosis and treatment of patients with vasospastic angina (coronary spastic angina) (JCS 2008): digest version. *Circ J,* 2010. 74(8): p. 1745-62.

[82] Okumura, K., et al., Sensitivity and specificity of intracoronary injection of acetylcholine for the induction of coronary artery spasm. *J Am Coll Cardiol,* 1988. 12(4): p. 883-8.

[83] Galiuto, L., et al., Reversible coronary microvascular dysfunction: a common pathogenetic mechanism in Apical Ballooning or Tako-Tsubo Syndrome. *Eur Heart J,* 2010. 31(11): p. 1319-27.

[84] Gibson, C.M., et al., TIMI frame count: a quantitative method of assessing coronary artery flow. *Circulation,* 1996. 93(5): p. 879-88.

[85] Bybee, K.A., et al., Clinical characteristics and thrombolysis in myocardial infarction frame counts in women with transient left ventricular apical ballooning syndrome. *Am J Cardiol,* 2004. 94(3): p. 343-6.

[86] Elesber, A., et al., Myocardial perfusion in apical ballooning syndrome correlate of myocardial injury. *Am Heart J,* 2006. 152(3): p. 469 e9-13.

[87] Meimoun, P., et al., Transient impairment of coronary flow reserve in tako-tsubo cardiomyopathy is related to left ventricular systolic parameters. *Eur J Echocardiogr*, 2009. 10(2): p. 265-70.

[88] Kume, T., et al., Assessment of coronary microcirculation in patients with takotsubo-like left ventricular dysfunction. *Circ J,* 2005. 69(8): p. 934-9.

[89] Abe, Y., et al., Assessment of clinical features in transient left ventricular apical ballooning. *J Am Coll Cardiol*, 2003. 41(5): p. 737-42.

[90] Kawai, S., et al., Ampulla cardiomyopathy ('Takotusbo' cardiomyopathy)--reversible left ventricular dysfunction: with ST segment elevation. *Jpn Circ J*, 2000. 64(2): p. 156-9.

[91] Mori, H., et al., Increased responsiveness of left ventricular apical myocardium to adrenergic stimuli. *Cardiovasc Res*, 1993. 27(2): p. 192-8.

[92] Kawano, H., R. Okada, and K. Yano, Histological study on the distribution of autonomic nerves in the human heart. *Heart Vessels*, 2003. 18(1): p. 32-9.

[93] Lathers, C.M., R.M. Levin, and W.H. Spivey, Regional distribution of myocardial beta-adrenoceptors in the cat. *Eur J Pharmacol*, 1986. 130(1-2): p. 111-7.

[94] Heather, L.C., et al., Isoproterenol induces in vivo functional and metabolic abnormalities: similar to those found in the infarcted rat heart. *J Physiol Pharmacol,* 2009. 60(3): p. 31-9.

[95] Mantravadi, R., et al., Autonomic nerve stimulation reverses ventricular repolarization sequence in rabbit hearts. *Circ Res*, 2007. 100(7): p. e72-80.

[96] Khoueiry, G., et al., Reverse Takotsubo cardiomyopathy in the setting of anaphylaxis treated with high-dose intravenous epinephrine. *J Emerg Med,* 2013. 44(1): p. 96-9.

[97] Collen, J., W. Bimson, and P. Devine, A variant of Takotsubo cardiomyopathy: a rare complication in the electrophysiology lab. *J Invasive Cardiol,* 2008. 20(11): p. E310-3.

[98] Bonnemeier, H., et al., "The artichoke heart": the inverse counterpart of left ventricular apical ballooning. *Resuscitation*, 2007. 72(3): p. 342-3.

[99] Song, B.G., et al., The clinical characteristics, laboratory parameters, electrocardiographic, and echocardiographic findings of reverse or inverted takotsubo cardiomyopathy: comparison with mid or apical variant. *Clin Cardiol,* 2011. 34(11): p. 693-9.

[100] Ramaraj, R. and M.R. Movahed, Reverse or inverted takotsubo cardiomyopathy (reverse left ventricular apical ballooning syndrome) presents at a younger age compared with the mid or apical variant and is always associated with triggering stress. *Congest Heart Fail*, 2010. 16(6): p. 284-6.

[101] Brewington, S.D., et al., Reproducible microvascular dysfunction with dobutamine infusion in Takotsubo cardiomyopathy presenting with ST segment elevation. *Catheter Cardiovasc Interv*, 2006. 68(5): p. 769-74.

[102] Whalen, E.J. and S.J. Lewis, In vivo evidence that isoproterenol may increase heart rate in the rat by mechanisms in addition to activation of cardiac beta(1)- or beta(2)-adrenoceptors. *Eur J Pharmacol*, 1999. 382(3): p. 207-10.

[103] Thawornkaiwong, A., S. Preawnim, and J. Wattanapermpool, Upregulation of beta 1-adrenergic receptors in ovariectomized rat hearts. *Life Sci,* 2003. 72(16): p. 1813-24.

[104] Bristow, M.R., et al., Beta 1- and beta 2-adrenergic-receptor subpopulations in nonfailing and failing human ventricular myocardium: coupling of both receptor subtypes to muscle contraction and selective beta 1-receptor down-regulation in heart failure. *Circ Res,* 1986. 59(3): p. 297-309.

[105] Ishikura, F., Y. Takano, and T. Ueyama, Acute effects of beta-blocker with intrinsic sympathomimetic activity on stress-induced cardiac dysfunction in rats. *J Cardiol,* 2012. 60(6): p. 470-4.

[106] Takano, Y., T. Ueyama, and F. Ishikura, Azelnidipine, unique calcium channel blocker could prevent stress-induced cardiac dysfunction like alpha.beta blocker. *J Cardiol*, 2012. 60(1): p. 18-22.

[107] Ebert, S.N., et al., Expression of phenylethanolamine n-methyltransferase in the embryonic rat heart. *J Mol Cell Cardiol*, 1996. 28(8): p. 1653-8.

[108] Ebert, S.N. and D.G. Taylor, Catecholamines and development of cardiac pacemaking: an intrinsically intimate relationship. *Cardiovasc Res*, 2006. 72(3): p. 364-74.

[109] Ebert, S.N. and R.P. Thompson, Embryonic epinephrine synthesis in the rat heart before innervation: association with pacemaking and conduction tissue development. *Circ Res*, 2001. 88(1): p. 117-24.

[110] Huang, M.H., et al., An intrinsic adrenergic system in mammalian heart. *J Clin Invest,* 1996. 98(6): p. 1298-1303.

[111] Papka, R.E., A study of catecholamine-containing cells in the hearts of fetal and postnatal rabbits by fluorescence and electron microscopy. *Cell Tissue Res,* 1974. 154(4): p. 471-84.

[112] Elayan, H.H., B.P. Kennedy, and M.G. Ziegler, Cardiac atria and ventricles contain different inducible adrenaline synthesising enzymes. *Cardiovasc Res*, 1990. 24(1): p. 53-6.

[113] Armour, J.A., et al., Gross and microscopic anatomy of the human intrinsic cardiac nervous system. *Anat Rec*, 1997. 247(2): p. 289-98.

[114] Pierpont, G.L., E.G. DeMaster, and J.N. Cohn, Regional differences in adrenergic function within the left ventricle. *Am J Physiol*, 1984. 246(6 Pt 2): p. H824-9.

[115] Angelakos, E.T., Regional Distribution of Catecholamines in the Dog Heart. *Circ Res*, 1965. 16: p. 39-44.

[116] Clarke, G.L., et al., ss-adrenoceptor blockers increase cardiac sympathetic innervation by inhibiting autoreceptor suppression of axon growth. *J Neurosci,* 2010. 30(37): p. 12446-54.

[117] Lau, C., S.P. Burke, and T.A. Slotkin, Maturation of sympathetic neurotransmission in the rat heart. IX. Development of transsynaptic regulation of cardiac adrenergic sensitivity. *J Pharmacol Exp Ther*, 1982. 223(3): p. 675-80.

[118] Habecker, B.A., N.M. Malec, and S.C. Landis, Differential regulation of adrenergic receptor development by sympathetic innervation. *J Neurosci,* 1996. 16(1): p. 229-37.

[119] Ebert, S.N., et al., Catecholamine-synthesizing cells in the embryonic mouse heart. *Ann N Y Acad Sci*, 2008. 1148: p. 317-24.

[120] Kloberg, A.J. and R. Fritsche, Catecholamines are present in larval Xenopus laevis: a potential source for cardiac control. *J Exp Zool*, 2002. 292(3): p. 293-303.

[121] Kimura, K., M. Ieda, and K. Fukuda, Development, maturation, and transdifferentiation of cardiac sympathetic nerves. *Circ Res*, 2012. 110(2): p. 325-36.

[122] Slavikova, J., et al., Catecholaminergic neurons in the rat intrinsic cardiac nervous system. *Neurochem Res,* 2003. 28(3-4): p. 593-8.

[123] Spurgeon, H.A., et al., Catecholamines associated with conductile and contractile myocardium of normal and denervated dog hearts. *J Pharmacol Exp Ther*, 1974. 190(3): p. 466-71.

[124] Ellison, J.P. and R.G. Hibbs, Catecholamine-containing cells of the guinea pig heart: an ultrastructural study. *J Mol Cell Cardiol*, 1974. 6(1): p. 17-26.

[125] Padbury, J.F., et al., Ontogenesis of tissue catecholamines in fetal and neonatal rabbits. *J Dev Physiol,* 1981. 3(5): p. 297-303.

[126] Abrahamsson, T., A.C. Jonsson, and S. Nilsson, Catecholamine synthesis in the chromaffin tissue of the African lungfish, Protopterus aethiopicus. *Acta Physiol Scand*, 1979. 107(2): p. 149-51.

[127] Forsgren, S., M. Moravec, and J. Moravec, Catecholamine-synthesizing enzymes and neuropeptides in rat heart epicardial ganglia; an immunohistochemical study. *Histochem J,* 1990. 22(12): p. 667-76.

[128] Dail, W.G., Jr. and G.C. Palmer, Localization and correltion of catecholamine-containing cells with adenyl cyclase nd phosphodiesterase activities in the human fetal heart. *Anat Rec*, 1973. 177(2): p. 265-87.

[129] Krizanova, O., et al., Existence of cardiac PNMT mRNA in adult rats: elevation by stress in a glucocorticoid-dependent manner. *Am J Physiol Heart Circ Physiol*, 2001. 281(3): p. H1372-9.

[130] Kvetnansky, R., et al., Localization and regulation of phenylethanolamine N-methyltransferase gene expression in the heart of rats and mice during stress. *Ann N Y Acad Sci*, 2004. 1018: p. 405-17.

[131] Legriel, S., et al., Recurrent takotsubo cardiomyopathy triggered by convulsive status epilepticus. *Neurocrit Care*, 2008. 9(1): p. 118-21.

[132] Behr, E.R. and S. Mahida, Stress cardiomyopathy and the acquired long QT syndrome. *Heart Rhythm,* 2011. 8(4): p. e1; author reply e1-2.

[133] Al-Khatib, S.M., et al., What clinicians should know about the QT interval. *JAMA*, 2003. 289(16): p. 2120-7.

[134] Valbusa, A., et al., Takotsubo cardiomyopathy and torsade de pointes in myasthenic crisis: be aware of QT prolongation. *Am J Emerg Med,* 2013. 31(12): p. 1717-8.

[135] Kurisu, S., et al., Torsade de pointes associated with bradycardia and takotsubo cardiomyopathy. *Can J Cardiol*, 2008. 24(8): p. 640-2.

[136] Rotondi, F. and F. Manganelli, Takotsubo cardiomyopathy and arrhythmic risk: the dark side of the moon. *Eur Rev Med Pharmacol Sci*, 2013. 17(1): p. 105-11.

[137] Salazar-Mendiguchia, J., et al., An unusual complication of a Takotsubo cardiomyopathy: a not so benign disease? *Int J Cardiol*, 2011. 150(3): p. 348-9.