Spatial control of the βAR system in heart failure: the transverse tubule and beyond

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Abstract

The β1-adrenoceptors (β1AR) and beta-2 (β2AR) adrenoceptors represent the predominant pathway for sympathetic control of myocardial function. Diverse mechanisms have evolved to translate signalling via these two molecules into differential effects on physiology. In this review, we discuss how the functions of the βAR are organized from the level of secondary messengers to the whole heart to achieve this. Using novel microscopy and bio-imaging methods researchers have uncovered subtle organization of the control of cyclic adenosine monophosphate (cAMP), the predominant positively inotropic pathway for the βAR. The β2AR in particular is demonstrated to give rise to highly compartmentalized, spatially confined cAMP signals. Organization of β2AR within the T-tubule and caveolae of cardiomyocytes concentrates this receptor with molecules which buffer and shape its cAMP signal to give fine control. This situation is undermined in various forms of heart failure. Human and animal models of heart failure demonstrate disruption of cellular micro-architecture which contributes to the change in response to cardiac βARs. Loss of cellular structure has proved key to the observed loss of confined β2AR signalling. Some pharmacological and genetic treatments have been successful in returning failing cells to a more structured phenotype. Within these cells it has been possible to observe the partial restoration of normal β2AR signalling. At the level of the organ, the expression of the two βAR subtypes varies between regions with the β2AR forming a greater proportion of the βAR population at the apex. This distribution may contribute to regional wall motion abnormalities in Takotsubo cardiomyopathy, a syndrome of high sympathetic activity, where the phosphorylated β2AR can signal via G protein to produce negatively inotropic effects.

Keywords

T-tubules • Cardiomyocyte • Beta-adrenergic receptors • Heart failure • Sympathetic system • Cardiac

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1. βAR signalling compartmentation in cardiac myocytes

The β1 and β2 adrenergic receptors (βARs) act through their coupling to G proteins and to the production of the common second messenger cyclic adenosine monophosphate (cAMP) that controls the catecholamine-dependent changes in rate, force, and speed of contraction of the heart. However, selective stimulation of these two receptor subtypes leads to clearly distinct physiological and pathophysiological responses. In healthy cardiac myocytes, β1ARs, but not β2ARs, stimulate the cAMP-dependent protein kinase (PKA)-mediated phosphorylation of a number of key proteins in heart contraction, including the L-type Ca2+ channels (LTCC), the sarcoplasmic reticulum Ca2+ release channel (RyR), phospholamban (relieving the sarcoplasmic reticulum Ca2+-ATPase inhibition) and cardiac contractile proteins.1,2 Through these effector pathways, the β1ARs produce positive inotropic and lusitropic responses. In adult rat or mouse cardiomyocytes, which have been extensively used to investigate compartmentalization, activation of β2AR leads only to LTCC phosphorylation with significantly smaller inotropic and no lusitropic effects.3 Heart-specific overexpression of β2AR in transgenic mice induces progressive cardiac hypertrophy and heart failure while overexpression of β2AR does not, at least until levels are high.4–6 Selective β2AR stimulation of isolated cardiomyocytes induces apoptosis, whereas β2AR stimulation shows an anti-apoptotic effect.7,8

βAR-subtype-specific differences in inotropy and lusitropy have been attributed to distinct patterns of cAMP compartmentation observed. Early research involving cell fractionation9 and ion channel recordings in two distant parts of a single cardiomyocyte10 showed that the activation of cardiomyocytes typically results in raised CAMP levels in distinct

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subcellular compartments that lead to localized LTCC activation. In ventricular rat cardiomyocytes, Chen-Izu et al.\textsuperscript{11} have provided evidence that $\beta_2$AR signalling to the LTCC is via a diffusive pathway through the cytosol, whereas $\beta_1$AR signalling is localized to the cell membrane. The field has been greatly enhanced by studies using electrophysiological and fluorescent biosensors to report cAMP levels in precise compartments.\textsuperscript{12–15}

2. Role of transverse tubules in $\beta$AR signalling compartmentation

One potential source of compartmentation in the highly structured ventricular cardiomyocyte is the physical arrangement of the sarcolemma. The most evident features are the transverse tubules (T-tubules), a network of deep invaginations of the surface membrane which form an interconnected network of tubes penetrating deep into the interior of the cardiomyocyte. These tubules have openings within the z-groove, $\sim$250 nm at their entrance. T-tubules conduct the electrical impulse into the centre of the myocyte and allow the trigger Ca\textsuperscript{2+} (entering by the L-type Ca\textsuperscript{2+} channel) to closely approach the sarcoplasmic reticulum Ca\textsuperscript{2+} release channel.\textsuperscript{16} T-tubules are shown to accumulate a number of molecules that are important in $\beta$AR signalling, such as the PDE4, PKA, AKAPs (PKA scaffolding proteins), and adenylyl cyclase (AC), many in a signalosome with the LTCC.\textsuperscript{16–18}

Although $\beta$AR localization by immunolabelling can be difficult, because of the low expression levels, monitoring $\beta$AR-dependent cAMP signalling in dynamics in living cells in real time was made possible by introducing highly sensitive fluorescent biosensors. These probes are based on fluorescence resonance energy transfer (FRET) between, for example, CFP and YFP fused to a single cyclic nucleotide-binding domain for cAMP from Epac, PKA, or the HCN channel.\textsuperscript{14,19} Using transgenic mice expressing a fluorescent sensor in cardiac myocytes, it was demonstrated that $\beta_2$AR-mediated cAMP signals propagated throughout large parts of the cell, whereas $\beta_1$AR signals were locally confined.\textsuperscript{18} To investigate the role of the T-tubule in this compartmentation, we developed a new nanoscale imaging technique which is a combination of FRET-based cAMP imaging with the scanning ion conductance microscopy (SICM) to study functional localization of $\beta$ARs. SICM is a non-optical method which uses a nanopipette as a scanning probe to image the surface topography of living cells and allows the resolution of the surface structural features of cardiac myocytes, such as z-grooves and T-tubules, with a resolution equal to the pipette’s inner diameter, typically $\sim$20–50 nm.\textsuperscript{20,21} After the acquisition of the cell-surface topography, we precisely position the pipette onto various membrane regions of defined morphology to locally apply agonists and antagonists of the $\beta$ARs. Local stimulation is achieved by applying pressure into the pipette while constantly superfusing the cells with the buffer/antagonist solution from the side.\textsuperscript{22} We measured cAMP synthesis using the sensitive cAMP sensor Epac2-camps after application of agonist either in the T-tubule or on the crest of the cell.\textsuperscript{19} Selective stimulation of $\beta_2$ARs in both regions resulted in a robust decrease in FRET, reflecting the stimulation of cAMP synthesis by the receptors localized in different parts of the membrane. In contrast, $\beta_1$AR selective stimulation led to cAMP signals only in the T-tubules, but not in the other regions of the sarcolemma.

We asked whether the absence of the $\beta_2$AR-cAMP signal on the crest of the cell may result from higher rates of local cAMP degradation by phosphodiesterases (PDEs)\textsuperscript{12} or from coupling of the $\beta_1$AR to G-proteins in this particular compartment.\textsuperscript{23,24} Inhibition of PDEs with 3-isobutyl-1-methylxanthine led to slightly higher cAMP levels in the T-tubules but did not produce any signals in the outer compartments. Likewise, G-protein inhibition with pertussis toxin (PTX) did not change the pattern of the $\beta_2$AR signal localization. These data indicate that in normal cardiac cells $\beta_2$AR-cAMP signalling is exclusively localized to the T-tubules, whereas $\beta_1$ARs are present in both the crests of the cells and the T-tubules. The functional consequence of the localization of $\beta_2$ARs was the lack of propagation of cAMP produced by this subtype, whereas cAMP from the $\beta_1$AR (even when produced at similar levels to that of the $\beta_2$AR) was observed to spread throughout the cardiomyocyte. A wider range of phosphorylation targets is therefore available to $\beta_1$AR-dependent cAMP.

We then suggested that cAMP buffering by the cAMP-dependent PKA might contribute to the localized $\beta_2$AR-cAMP signalling in the T-tubules. Cardiac myocytes express two isoforms of PKA: PKA-RI and PKA-RII, which localize to different subcellular compartments.\textsuperscript{25} Soluble fraction of myocyte lysates mainly contains PKA-RI, whereas PKA-RII is mainly found in the particulate fraction. Recently it has been shown that PKA-RI is anchored to specific subcellular sites.\textsuperscript{25} Compartmentation of PKAs helps to bring them in proximity of their targets thus favouring selective phosphorylation of these targets. Di Benedetto et al.\textsuperscript{25} used targeted cAMP sensors: either RI\textsubscript{epac} or RI\textsubscript{II_epac} and found that the activation of $\beta$ARs produces a pool of cAMP that preferentially activates PKA-RII over PKA-RI. After immunostaining for the PKA-RII, we found a striated localization pattern in healthy cardiomyocytes\textsuperscript{22} which suggest that it may be localized into periodic structures. When we disrupted the t-tubular localization of the PKA by the Ht31 peptide, which blocks the interaction between RII subunits and A-kinase anchoring proteins\textsuperscript{15,26} we observed that $\beta_2$AR-cAMP signals propagated throughout larger parts of the cell. This finding suggests that the cAMP produced upon stimulation of $\beta_2$ARs in the T-tubules may be locally confined by the interaction with localized PKA-RII molecules enriched in this structural compartment.\textsuperscript{22}

3. Involvement of caveolae in $\beta$AR signalling compartmentation

Apart from T-tubules, there are smaller invaginations (\~\~50 nm diameter) known as caveolae which are distributed across the surface of CMs. Many of the initial studies on $\beta_1$AR/$\beta_2$AR compartmentation were performed in neonatal rat cardiomyocytes and concentrated on the role of caveolae.\textsuperscript{27–29} Neonatal myocytes do not have T-tubules, but have a higher density of caveolae than adult, and it has been suggested that caveolae may be developmental precursors of T-tubules and share some of the same functions.\textsuperscript{30} Caveolin-3 (Cav3) is the principal protein component of cardiac caveolae: several studies using transgenic mice overexpressing Cav3 revealed dramatic increases in the number of sarcolemmal muscle cell caveolae.\textsuperscript{31} Overexpression of Cav3 in Duchene muscular dystrophy skeletal myofibres increased caveolae number.\textsuperscript{32} The development of the T-tubules in striated muscle appears to depend on cholesterol and Cav3 and shows similarities to the development of caveolae.\textsuperscript{33} Concentrations of caveolin/caveolae have been noted within the t-tubular domain.\textsuperscript{34} Double Cav1/Cav3 knockout mice showed complete loss of caveolae, with associated t-tubular disorganization and dramatic cardiomyopathy.\textsuperscript{35,36}
Caveolae have been shown to contain a concentration of signalling components of which some, including Gi and eNOS, would be predicted to either reduce or oppose cAMP-dependent signalling. In neonatal cardiomyocytes, β2ARs were found in various membrane fractions, but β2ARs were reported to localize predominantly in caveolae and to interact with G-proteins due to this spatial localization (although AC was also present in this domain). Recent evidence in adult rat ventricular myocytes indicates that cholesterol depletion disrupted β2AR coupling to G-proteins and acute chemical caveolae disruption using methyl-β-cyclodextrin (MβCD) led to modification of β2-AR- and β2AR-mediated cAMP responses. Disruption of caveolae increased β2AR-mediated cell contractility and cAMP accessed the PKA compartment more effectively increasing PKA activity. Previously, using the SICM/FRET technique we showed that chemical cholesterol depletion in cardiomyocytes using MβCD results in redistribution of the β2AR-cAMP signalling from the T-tubules to non-tubular outer membrane areas, where this receptor was able to trigger far-reaching cAMP signals. This raises the possibility that confinement of the β2AR in caveolin-rich domains contributes to the silencing of the cAMP signal and the preferential coupling to other pathways.

4. Remodelling of cardiomyocyte structure and β2AR redistribution in animal models of heart failure

Blunting of response to βAR stimulation has been a consistent finding across numerous experimental small and large animal models of heart failure. These observations support the hypothesis that decreased βAR responsiveness is part of a common pathway or signature of a failing heart, irrespective of aetiology [post-myocardial infarction, transaortic constriction (TAC), anthracycline, tachycardic pacing, genetic]. This is critical element for experimental design and phenotyping experimental ‘heart failure’ models. For example, myocardial infarction induced by surgical coronary ligation is a common model employed in the cardiovascular research community. Many studies have demonstrated that, like humans, the remaining viable myocardium is initially hypercontractile, and surviving ventricular cardiomyocytes transition via a pathological hypertrophic phenotype. Insights from cardiomyocyte development are helpful, and loss of T-tubules is also reproducible across the animal species and heart failure models studied. This allows studies to evaluate the mechanisms, timecourse, and functional sequelae of T-tubule loss in a manner not possible in human cardiomyocytes. Indeed, the first description of T-tubule loss in acquired heart failure was reported in the canine model. Studies using cardiomyocytes isolated from various rodent heart models have demonstrated a significant disruption of excitation–contraction coupling and stimulated calcium transients correlated to the location of T-tubule loss. Cardiomyocytes from failing spontaneous hypertensive rat hearts demonstrated a temporal delay in excitation–contraction coupling related to increased spatial separation of the junctional SR from the T-tubule membrane, generating ‘orphaned ryanodine receptors’ with an associated increase in spontaneous Ca2+ release events. T-tubules are reduced during the development of left ventricular hypertrophy after aortic banding, with the discrete local loss and global re-organization of the T-tubule system preceding overt left ventricular dysfunction. Further T-tubule disruption and loss developed with progression to advanced chronic heart failure. The matched observations in large animal models of heart failure are consistent with the hypothesis that T-tubule loss is part of a common pathway in the failing myocardium, irrespective of aetiology, or species.

Surface scans of the failing cardiomyocytes using SICM showed that there were structural changes beyond the T-tubule to include loss of the surface membrane trough, the z-groove, so-called due to their anatomical alignment to the Z line of the sarcomere. The loss of z-grooves and T-tubule openings, measured using SICM, was associated with regional repositioning of functional βARs. We showed that β2AR-cAMP signal localization was disturbed in a rat model of myocardial infarction. In contrast to healthy cells, cAMP signal was observed to propagate along the cardiomyocyte even after local β2ARs have been stimulated specifically in T-tubules. This suggests that in heart failure β2AR-cAMP signalling relocates from the T-tubule area to the crest area. Strikingly, stimulation of β2ARs in de-tubulated areas of failing cardiomyocytes produced diffuse cAMP signalling that propagated throughout the entire cytosol, similar to the β1AR signal. Immunostaining of the PKA-RII revealed a loss of the striated PKA localization pattern in heart failure cells. On the basis of our experimental data, we propose a working model of how the changes seen in heart failure may occur (Figure 1). We propose that redistribution of the β2AR-cAMP signalling from the T-tubules to the cell crest in failing cardiomyocytes and the simultaneous loss of PKA localization results in uncoupling of the β2ARs from the localized pools of PKA that are responsible for the compartmentation of β2AR-cAMP.

The mechanism(s) underlying T-tubule loss are multiple and complex. Insights from cardiomyocyte development are helpful, and these have demonstrated that membrane invagination and tubulogenesis, plus interlocking connections with the adjacent sarcoplasmic reticulum membrane to stabilize Ca2+ release units, are critical for T-tubule development. A number of proteins have been identified which appear to play critical roles in tubulogenesis in the developing heart. BIN1, also known as amphiphysin-2, directly binds to membrane phospholipids and BIN1 overexpression in skeletal muscle-derived cell lines induces formation of membrane tubules. BIN1 may also serve to anchor microtubules to the T-tubule system, a necessary interaction for the physical targeting of LTCC to the T-tubule orifice. TCAP is a protein anchor and biomechanical transducer.
between the myofilament and z disc macromolecular complex and TCAP depletion in a zebrafish model leads to impaired transverse tubulogenesis during development. Junctophilin 2 (JP2) has been proposed as a critical regulator of the T-tubule-SR junctional anatomy, fulfilling a protein docking role. Loss of JP2 has been observed where measured in various models of chronic heart failure, associated with the loss of T-tubules. However, discrepancies exist regarding the role of these various structural proteins in recovery (see below).

5. Recovery of T-tubule structure during reverse remodelling and βAR signalling

It is conceivable that T-tubule structural integrity is in a dynamic equilibrium between synthesis and degradation. During the development of the foetal and neonatal mammalian heart the balance favours synthesis, and in the adult healthy heart there is equilibrium to maintain a steady T-tubule system. With increased mechanical strain, oxidative stress and hereto unidentified factors activated following cardiac injury, the balance favours degradation over synthesis, resulting in progressive loss of T-tubule density and organization.

Consistent with this concept of plasticity, we and others have also shown the recovery of T-tubule density and cardiomyocyte surface membrane topology, following recovery and reverse remodelling of the chronically failing heart. We used SERCA2a gene therapy to rescue our chronic rat heart failure model in an energetically favourable manner and detected a recovery of T-tubule density as well as surface z-groove anatomy as quantified by the z-groove index. This recovery was associated with spatial resynchronization of stimulated calcium release, with a reduction in the number of delayed release sites, and recovery of βAR sensitivity as assessed using isoprenaline concentration-response studies on myocyte contraction. Using combined SICM-FRET, we identified that the recovery of the T-tubules and z-groove architecture was associated with correct sarcolemmal location of βARs. Specifically, the β2AR-cAMP signal on the crest was reduced, and did not propagate through the cell, with a correlation between the recovery of z-groove structure and the extent of β2AR relocalization (Figure 2). T-tubule plasticity is not restricted to the left ventricular cardiomyocytes, with Xie et al. reporting partial restoration of T-tubules in right ventricular cardiomyocytes from a rat model of pulmonary artery hypertension after treatment with sildenafil. The molecular mechanisms underpinning this reverse remodelling remain to be determined, but consistently the recovery of BIN1 expression, reduced in chronic failing myocardium, is reported. Other factors, including TCAP and JP2, show variable recovery, implying there may be redundancy in elements of the system, and/or differences between species and models.
6. How do the βAR/t-tubule changes in animal models relate to human heart failure?

Many of the key findings on animal models have been reproduced in the human heart, but there are some important differences. Both the loss of T-tubule density and flattening of the surface have been demonstrated in cardiomyocytes from the failing human heart using SICM or di-8-ANEPPS staining. In our study, similar changes were observed in cells from patients with ischaemic, idiopathic dilated, or hypertrophic cardiomyopathy, and were slightly greater in magnitude than alterations seen in the rat MI HF model. Values of the T-tubule index in the failing hearts were close to the 0.25 seen in a similarly mixed population of ischaemic cardiomyopathy, idiopathic dilated cardiomyopathy and congenital valvular disease. Hong et al. showed a less organized periodicity of the T-tubule structural protein BIV1 in human failing cardiomyocytes, and also noted a population of shallow (1–2 μm) T-tubules in addition to the sparse elongated ones running into the centre of the cell. Dilation of T-tubules and appearance of longitudinal elements (possibly subsarcolemmal and therefore undetectable with surface scanning by SICM) have also been observed.

The failing human heart is well known for desensitization of the βAR system, independent of aetiology, and it is accepted that there is loss of the β1AR with preserved β2AR number but β2AR ‘uncoupling’ (now possible to be interpreted as non-cAMP coupling). Signalling partners of the human β1- and β2ARs differ from those in the rodent heart in a number of respects, which are pertinent to βAR desensitization and compartmentation. Unlike rodent, PDE3 is predominant over PDE4 in the control of cAMP in the failing human heart. Coupling of the β2AR to Gi is seen in the normal human atria, but there is less evidence for this in ventricular muscle. Although β2AR-cAMP-mediated positive inotropism is strongly suppressed by Gi...
even in normal rat or mouse heart.3,76 the same is not true for the human ventricle.27 Here, the β2AR couples to cAMP production as strongly or even more strongly than β1AR,76 and phosphorylation of phospholamban, which is not observed in the rodent heart after β2AR stimulation, is equal to that produced by the β1AR.79 In the failing human heart, however, the βAR response is restored by PTX treatment, implying a Gi-mediated component to desensitization.80 Similarly β-blockers which can actively couple the β2AR to Gi have a PTX-sensitive negative inotropic effect in ventricular myocytes from the failing, not non-failing, human heart.81 Only in conditions of excess acute catecholamine exposure is there a possibility that Gi signalling can modulate contractile effects of βAR stimulation of human hearts not in end-stage failure: this is discussed further below in relation to Takotsubo cardiomyopathy (TCM).

Less is known for human than rodent regarding the compartmentation of βAR signalling in the ventricular myocyte, or its alteration in heart failure. Basal levels of phosphorylation are difficult to determine accurately in tissue sampled from end-stage failing human where there is a low but the residual level of βARs combined with a high sympathetic drive (and depend on the presence of clinical β-blockade). Inotropic support of brain-dead donors with catecholamines is also a confounder for control human samples. However, key components involved in spatial segregation are changed in the failing human heart: Gβγ and GRK2/GRK582,83 are up-regulated and PKA-AKAP associations are remodelled.84 Differential phosphorylation of PKA targets indicates an independent control of particular pools of cAMP, whereas phospholamban, protein phosphatase inhibitor-1, myosin-binding protein C, and troponin I85–88 are generally found to be hypophosphorylated in the failing myocardium, the opposite is true for RyR89 and the LTCC.90 The arrhythmogenic effect of catecholamines contributes to around half of all heart failure deaths, especially in patients with moderate rather than end-stage disease, and clinical trials show this to be increased by measures which raise cAMP but prevented by β-blockers.91,92 In ventricular myocytes from the failing human heart, maximum inotropic responses to isoprenaline, forskolin, or a cAMP analogue are limited by the appearance of arrhythmias.93 This implies that although the cAMP levels raise cAMP but prevented by β-blockers.91,92 In ventricular myocytes from the failing human heart, maximum inotropic responses to isoprenaline, forskolin, or a cAMP analogue are limited by the appearance of arrhythmias.93 This implies that although the cAMP levels

7. Spatial gradients of βAR signalling: beyond the single cell

Recently, we have explored a syndrome in which gradients of the βAR appear to underlie acute heart failure, and these gradients extend beyond the span of the T-tubule or even the individual cardiomyocyte. TCM, also known as stress cardiomyopathy, represents a singular syndrome distinct from other heart failure entities. TCM typically presents to emergency medical staff as an acute coronary syndrome, but subsequent coronary angiography reveals unobstructed coronary vessels and typically left ventricular apical hypokinesis.94This cardiac dysfunction causes a characteristic ballooning of the apex and acutely reduces the cardiac output, but in most cases reverses within days to weeks leading to full recovery of the patient. TCM is predominantly a disorder of post-menopausal women who have been subjected to an emotionally or physically distressing event. Circulating catecholamine levels are surprisingly high in TCM patients, even compared with sufferers of acute myocardial infarction95,96 and iatrogenic catecholamine exposure can also cause the syndrome.97 As the cellular receptors responsible for transducing sympatheo-adrenal signals into physiological responses, the myocardial βARs have a clear role in TCM.

We have hypothesized98 that the acute phase may be dependent upon the supraphysiological stimulation of β2AR by adrenaline, causing a switch in coupling to Gi. This results in a rapid negatively inotropic effect reducing the cardiac output, generating the acute heart failure state with associated symptoms, but potentially averting deleterious calcium overload, arrhythmias, and apoptotic or necrotic cell death. A recent study by our group demonstrated that high levels of adrenaline injected into anaesthetized rats reproduced this apical dysfunction. This was not replicated by noradrenaline injection, and was averted in animals pre-treated with PTX to remove the effects of Gβγ.65 Ueyama et al.99 were also able to evoke Takotsubo-like left ventricular changes in rats subjected to immobilization stress. Importantly, we showed that a gradient of β2ARs was responsible for the regional nature of the effect, with the β2AR to β1AR ratio higher at the apex of the heart than at the base. This is likely related to the higher sympathetic innervation (with the β1, AR-selective noradrenaline as the main neurotransmitter) at the base and the greater sensitivity of the apex to circulating adrenaline. Notably, attempting to prevent the negative inotropic effect through the block of β2AR and downstream signalling increased the rate of sudden death during the adrenaline challenge. This syndrome may provide a clue for the evolutionary origin of the distinct cardio-protective role of the β2AR and the necessity for compartmentation of its cAMP-dependent action.

8. Summary and further questions

βAR signalling through the cAMP system is spatially controlled in a subtype-dependent manner with the β1AR signal detected only after stimulation at the T-tubule. These experiments do not tell us whether the β1AR itself is absent from the non-tubule sarcosome, or whether it is present but preferentially coupled to a non-Gs-cAMP pathway. Silencing of the β2AR-cAMP signal would suggest a location of the β2AR within caveolin/cholesterol-rich domains, where it would associate with Gi or eNOS, and be consistent with increased β2AR-cAMP signals seen after disruption with MβCD. However, the evidence places those domains within the T-tubule rather than on the crest of the cardiomyocyte. Clearly, there is a need to establish the nature of the downstream signalling accessed by β2AR through Gi- or non-G-protein pathways and its spatial localization. During heart failure the β2AR-cAMP signalling is no longer localized to the T-tubule, and now propagates along the cardiomyocyte in similar way to that produced by the β1AR. Is this to partially replace the function of the preferentially lost β1ARs? Does it necessarily imply that non-Gs signalling of the β2AR is reduced? Does the loss of non-Gs coupling or the increased range of cAMP targets mean that the β2AR is now as pro-arrhythmic and pro-apoptotic as the β1AR? One point of note is that PKA-dependent phosphorylation of the β2AR drives the Gs to Gi switch72 and that a β2AR mutant without PKA phosphorylation sites resembled the β1AR in its damaging effect on the cardiomyocyte.72 Finally, reverse remodelling of the ventricular myocyte T-tubule system was observed after SERCA2a gene therapy and this was accompanied by a reversal of the
de-localization of the βAR. Does this indicate general recovery or is there a more specific link between SERCA2a levels and t-tubular structure or βAR signalling? In general, despite variations between rodent and human, the main components of the β1- and β2AR systems exist in both species and parallel changes in T-tubule architecture and βAR sensitivity are observed. It is therefore likely that the efforts put in by many groups to unravel the spatial regulation of the βAR system will be relevant for the understanding of human disease.

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