Multifaceted remodelling of cAMP microdomains driven by different aetiologies of heart failure

Kirstie A. De Jong¹,² and Viacheslav O. Nikolaev¹,²

¹ Institute of Experimental Cardiovascular Research, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
² German Center for Cardiovascular Research (DZHK), partner site Hamburg/Kiel/Lübeck, D-20246, Hamburg, Germany

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Correspondence
V. O. Nikolaev, Institute of Experimental Cardiovascular Research, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
Email: v.nikolaev@uke.de

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Heart failure with preserved ejection fraction (HFpEF) will soon take over as the predominant form of heart failure. This is largely driven by the continuing increased incidences of obesity and type 2 diabetes (T2D), which promote HFpEF in the absence of pressure overload stresses. With beta-blockers showing little effectiveness in treating obesity/T2D HFpEF and with no HFpEF-targeted drugs currently available, we are in urgent need of a better understanding of how obesity/T2D HFpEF develops and how we may treat this condition. An exciting emerging field aiming to do this focuses on the investigation of 3',5'-cyclic adenosine monophosphate (cAMP) microdomains in the heart. The previous work has largely focused on the investigation of cAMP microdomain remodelling in heart failure with reduced ejection fraction (HFrEF), with this work uncovering potential new targets for intervention strategies that otherwise would have been overlooked when studying changes in cAMP dynamics at the whole-cell level. In this review, we aimed to discuss current advancements in our understanding of cAMP microdomain remodelling in HFrEF vs that in obesity/T2D-associated HFpEF, with particular focus on the unresolved questions and limitations we face in being able to translate this knowledge.

Introduction
Cardiovascular disease accounts for 37% of all deaths within the European Union and costs the economy an estimated €210 billion per year [1]. While hypertension remains a driving force in the development of cardiovascular disease, obesity and type 2 diabetes (T2D) account for a growing burden on current and predicted cardiovascular cases, in particular due to these conditions increasing the risk of heart failure with preserved ejection fraction (HFpEF) [2,3]. Currently, 23% and 10% of people suffer from obesity and T2D, respectively. Of major concern in these patients is that traditional beta-blockers show little...
effectiveness in treating obesity/T2D-induced HFpEF [4], this is perhaps not surprising considering that beta-blockers were initially developed to treat heart failure with reduced ejection fraction (HFrEF), and it is only the increasing incidences of obesity and T2D and lack of HFpEF-specific drugs that have pushed their application into treating both forms of heart failure. With obesity and T2D incidence expected to double by the year 2080, we are in urgent need of a greater understanding of why current beta-blockers are not effective in treating obesity and T2D-induced HFpEF and how we may increase their effectiveness. An exciting emerging field in HFpEF research that aims to do this focuses on the investigation of cardiac microdomain remodelling of an essential second messenger in beta-adrenergic receptor (β-AR) signalling cascade, 3’,5’-cyclic adenosine monophosphate (cAMP). Previous work has largely focused on the investigation of cAMP microdomain remodelling in HFrEF, with this work uncovering dynamic remodelling events in cAMP microdomains that have aided in identifying potential new targets for intervention strategies that otherwise would have been overlooked when studying changes in cAMP dynamics at the whole-cell level [5,6]. In this review, we aimed to discuss our current understanding of cAMP compartmentation in HFrEF vs that in obesity and T2D-associated HFpEF, while paying close attention to some of the unresolved questions and obstacles that we face in being able to translate this knowledge from bench to bedside.

Notably, one of the first obstacles we faced in constructing this review was the lack of data available that had explored cardiac cAMP microdomains in obesity- and T2D-associated HFpEF. Indeed, in contrast to HFrEF research, there is a lack of data regarding cAMP microdomains in any aetiology of HFpEF, let alone specifically in obesity- and T2D-associated HFpEF; as such, this review centred on what is known about cAMP microdomains in the obese heart and T2D heart, in human tissue and rodent models that are suitable for HFpEF research.

What is heart failure?

When considering how cAMP microdomains are altered in HFrEF vs HFpEF, it is important to understand how these two forms of heart failure (HF) differ in terms of cardiac function. The criteria to diagnose HFrEF are clearly defined, characterised by the presence of systolic dysfunction with an ejection fraction <45–50%, with or without the accompanying presence of diastolic dysfunction. The criteria to diagnose HFpEF are less clear, although it is generally accepted to be characterised by the presence of diastolic dysfunction and elevated left ventricular (LV) filling pressure, without LV dilation and with preserved systolic function or mild systolic dysfunction (EF > 50%) [7,8]. Or simply put, HFrEF is a weak heart and HFpEF is a stiff heart. Both types of heart failure and their associated comorbidities and mortalities have been reported at a comparable prevalence [9–11]; however, HFpEF will soon take over as the predominant form of heart failure. While chronic hypertension drives both forms of heart failure, the increased burden of HFpEF that we are facing today in the future is largely driven by the increasing incidences of obesity and T2D, which can promote HFpEF in the absence of pressure overload stresses. Interestingly, both HFpEF and HFrEF are multifaceted diseases, each having multiple aetiological entities and distinct clinical subforms. Each of them requires better mechanistic understanding to provide more efficient therapies. One likely option is that each individual pathology has a characteristic pattern of cAMP microdomain remodelling [5,6], which we aim to better understand and carefully consider in this review.

What are cAMP microdomains?

cAMP is produced via β-AR signalling and inhibited via AMP-hydrolysing enzyme phosphodiesterases (PDEs), for which there are at least 5 families expressed in the heart (PDE1, PDE2, PDE3, PDE4 and PDE8) [12]. Increases in cAMP result in the activation of cAMP-dependent protein kinase (PKA) and Epac (exchange protein directly activated by cAMP), proteins which influence calcium influx and reuptake by activating calcium-handling proteins such as the L-type calcium channels (LTCCs), ryanodine receptors (RyRs) and the sarcoplasmic/endoplasmic reticulum (SR) calcium ATPase 2a (SERCA2a) [13,14]. These calcium-handling proteins are thought to be present in distinct microdomains within the cell, in which cAMP activity is regulated via the spatial sequestration of different β-ARs subtype signalling, A-kinase anchoring protein (AKAP) tethering of PKA and PDEs [6]. There are three known β-AR subtypes expressed in the heart that trigger cAMP production, the β1-AR, β2-AR and β3-AR. Catecholamine (namely epinephrine and norepinephrine) binding to these β-ARs induces a conformational change in the receptor allowing its coupling with heterotrimeric guanine nucleotide regulatory proteins (G proteins) [15]. Depending on the G protein that the β-AR couples with, cAMP production
will either be stimulated or inhibited, with Gαi coupling stimulating cAMP production via its interaction with adenylyl cyclase (AC), which catalyses the conversion of ATP to cAMP and Gαi coupling inhibiting cAMP production via acting as a negative regulator of Gαs signalling. All β-AR subtypes can couple with Gαs, while the β2-AR and β1-AR subtypes can both stimulate and inhibit cAMP production by coupling with both Gαs and Gαi [16,17].

To prevent sustained catecholamine stimulation of β-ARs, a negative feedback loop or ‘stop switch’ is initiated, in which β-ARs are phosphorylated by G protein-coupled receptor kinases (GRKs) and PKA. Phosphorylation uncouples the interaction of the β-AR with the G protein and induces either a degradation or internalisation of the β-AR. GRKs are suggested to only phosphorylate β-ARs with a bound agonist, while PKA is suggested to phosphorylate both agonist-bound and agonist-free β-ARs. Phosphorylation via GRKs induces a rapid, agonist-specific or ‘homologous desensitisation’ of β-ARs, in which phosphorylation of the agonist-occupied β-ARs promotes recruitment of a β-arrestin protein that prevents G protein binding, and this β-arrestin also acts as a scaffold protein for PDEs, allowing for a further decline of cAMP signalling [18]. While phosphorylation of β-ARs via PKA induces a conformational change in the β-ARs, inhibiting G protein binding is known as ‘heterologous’ or ‘non-agonist-specific’ desensitisation, as any stimulant that increases cAMP production may lead to the phosphorylation and desensitisation of β-ARs. Maintaining this balance between activation and inactivation of β-ARs is essential for normal cardiac function.

**Beta-adrenergic receptor microdomains in the healthy heart**

While all β-AR subtypes can stimulate cAMP production, the actions of these cAMP pools are divergent, and this is in part due to the formation of β-AR microdomains at the sarcolemma, where β-AR subtypes differ in their expression levels, subsarcolemmal distribution patterns and their coupling with AC isoforms, PDEs and the propagation of their cAMP signals (Fig. 1).

Considering β-AR expression levels and patterns first, in the human heart the β1-AR and β2-AR are expressed at a ratio of around 4:1 [19], while the β3-AR subtype is expressed at low levels. The β1-AR is distributed across the entire sarcolemma, and the β2-AR is confined to T tubules of the cardiomyocyte, specifically to caveolin-rich microdomains. The β1-AR is the main subtype responsible for inducing an inotropic response, with chronic stimulation of the β1-AR shown to induce cardiomyocyte apoptosis, hypertrophy and heart failure [20,21], while the β2-AR exhibits a lower ionotropic effect than the β1-AR and has been associated with exerting cardioprotective effects [22,23]. In humans, β2-AR polymorphisms are associated with adverse cardiovascular prognosis and decreased HF survival rates [24,25] and β2-AR knockout (KO) mice exhibit exacerbated responses to isoproterenol infusion, with increased death rates and cardiomyocyte apoptosis [26]. Interestingly, β3-AR is mostly coupled to Gαs and cAMP production in adipocytes, and it has been demonstrated to stimulate Gαs, nitric oxide and cyclic guanosine monophosphate (cGMP) synthesis in ventricular myocytes. This pathway has been reported to protect mouse hearts from isoproterenol-induced pathological remodelling and β3-AR expression is increased in HFrEF [27-29].

The two major AC isoforms expressed in the cardiomyocyte are AC5 and AC6. AC5 is localised predominantly to the T tubules, thereby allowing association with both the β1-AR and β2-AR, and AC6 is localised on the crest outside of the T tubules allowing association with the β2-AR [30]. AC5 KO mice have been reported to exhibit ~35-40% lower cAMP concentrations under basal conditions and in response to isoproterenol and forskolin stimulation. These mice exhibit blunted responses to isoproterenol-induced increases in left ventricular ejection fraction and are protected from transverse aortic constriction (TAC) and isoproterenol-induced heart failure [31,32]. On the other hand, AC6 overexpression protects from pressure overload-induced heart failure [33] and AC6 KO mice exhibit increased susceptibility to pressure overload and catecholamine-induced heart failure. These mice show no differences in basal cAMP levels but a reduction of 60–70% in isoproterenol-stimulated cAMP levels [34-36]. Indeed, the greater loss in isoproterenol-stimulated cAMP levels in AC6 KO mice than that in AC5 KO mice is consistent with the observation that AC6 but not AC5 regulates β-AR signalling in heart [37,38] and support the selective coupling of AC6 with the β1-AR on the crest of the sarcolemma (as this is the β-AR subtype responsible for inducing the main inotropic response).

Selective stimulation of the β1-AR and β2-ARs has been shown to induce different propagation of cAMP signals. Scanning ion conductance microscopy (SICM, which identifies the T tube and crest by producing a detailed topographical image of the cell surface), fluorescence resonance energy transfer (FRET) and a cAMP biosensors targeted to the cytoplasm (Epac2-
camps and HCN2-camps) have been utilised to directly monitor the spatial and temporal distribution of cAMP in cardiomyocytes in response to selective β1-AR and β2-AR stimulation [39,40]. This approach could identify that β1-AR-induced cAMP production resulted in far-reaching cAMP signals originating from the T tubules and crest, while β2-AR-induced cAMP signals were half that of the β1-AR and remained locally confined, originating from the T tubules. Interestingly, these differences in cAMP levels and propagation are likely accounted by activity of PDEs coupled to the β2-AR-stimulated cAMP pools and not due to β2-AR coupling with Gαi. As inhibition of PDE3 and PDE4, but not inhibition of Gαi, was able to increase β2-AR-stimulated cAMP levels to that of β1-AR stimulation. PDEs can therefore act to confine the level and propagation of β2-AR-cAMP signals [40]. These larger diffusion patterns of β1-AR signalling partly explain the divergent actions of β-AR subtype signalling and provide insight into how β1-AR signalling but not β2-AR signalling is able to activate far-reaching calcium-handling proteins producing a large ionotropic response.

Combined, these data suggest that the formation of β-AR microdomains assist in mediating divergent β-AR subtype-specific cAMP signalling.

**Beta-adrenergic receptor microdomains in HFrEF**

β-AR microdomains exhibit remodelling in early and late stages of HFrEF, with altered β-AR expression levels and localisation patterns, coupling with AC isoforms and PDEs and propagation of cAMP signals (Fig. 2).

Stimulation of β-ARs is increased in HFrEF due to chronic activation of the sympathetic nervous system, in which endogenous agonists, epinephrine and norepinephrine stimulate in excess in an attempt to meet increased contractility demands. To compensate for elevated sympathetic drive, GRK activity is increased. While initially protective, chronic activation of GRK leads to desensitisation of β-ARs (homologous desensitisation) and a blunted inotropic response [41,42]. This is evident from increased GRK2 expression and decreased β1-AR expression and function in HFrEF [43–45], with β1-AR downregulation occurring early [46] and correlating with disease severity [47–49]. In mice, chronic administration of isoproterenol increased GRK2 expression and induced β-AR desensitisation and hypertrophy [50], and reductions in GRK2 expression in the heart protected against myocardial infarction and TAC induced systolic dysfunction and myocardial fibrosis [51,52].

**Fig. 1.** 3′,5′-cyclic adenosine monophosphate (cAMP) microdomains in healthy cardiomyocytes. Depicting the regulation of cAMP within the beta-adrenergic receptor (β-AR) microdomains in which cAMP signals stimulated via the β1-AR are far-reaching and regulated via the phosphodiesterases (PDEs), PDE2, PDE3 and PDE4, while the β2-AR signals are locally confined and regulated via PDE3 and PDE4. In the L-type calcium channel (LTCC) microdomain, cAMP pools are locally regulated via β2-AR signalling and PDE3 and PDE4. In the ryanodine receptor (RyR) microdomain, cAMP pools are regulated via β1-AR signalling and PDE4, and in the phospholamban/sarcoplasmic endoplasmic reticulum, calcium ATPase 2a (PLN/SERCA2a) microdomain, PKA, is tethered by A-kinase anchoring protein 18 (AKAP186) and cAMP pools are regulated via PDE3 and PDE4.
hypertrophy and preserved cardiac reserve [51,52]. Compounding the negative effects of β₁-AR desensitisation further, an uncoupling between the remaining β-ARs with Gₐ₅ has been observed along with an increased expression and association of the β₂-AR with the Gₐ𝑖 subunit [53].

Further blunting β-AR signalling is brought about by an uncoupling between β-ARs and ACs in HFrEF, with reduced β-AR-stimulated AC catalytic activity and AC expression reported. In human failing left ventricular tissue, isoproterenol-induced activation of AC was found to be reduced by 45% compared with that in nonfailing donor tissue. This was accompanied by a reduction in isoproterenol-induced contraction of 53–73%, highlighting the functional defects reduced AC activity has on the myocardium [43]. A relationship between disease severity and AC uncoupling was uncovered in dogs, where a pacing-induced mild congestive heart failure resulted in reduced β-AR-stimulated AC activity but did not alter AC mRNA levels. These hearts already exhibited β-AR desensitisation and increased GRK activity and expression. A decrease in AC6 mRNA expression was only observed in response to pacing-induced severe congestive heart failure [49]. This is supported by another study identifying reduced AC6 mRNA expression 6 weeks post-TAC, with no change in AC mRNA expression detected earlier despite the presence of a reduced EF% < 50%. These data suggest that reduced β-AR-stimulated AC activity in HFrEF is not initially mediated by a downregulation of AC6 expression but rather a decrease in activity. However, it is still unclear from these studies whether there are any changes in AC6 expression at the protein level. Interestingly, while AC6 expression has been reported to be reduced in response to HFrEF, AC5 expression has been reported to be increased [54]. This matches the phenomenon often observed in HFrEF where the heart returns to foetal state (as AC5 is expressed at higher levels than AC6 in the neonatal heart). However, this increase in AC5 expression appears to be insufficient to maintain cardiac function and compensate for the decreased AC6 activity, suggesting AC6 activity is integral in maintaining normal cardiac function. Indeed, increasing β-AR responsiveness and cAMP stimulation via AC6 modulation has been shown to be beneficial in human and mouse HFrEF, with AC6 gene transfer increasing LV function in humans with HFrEF [55] and cardiac overexpression of AC6 increasing catecholamine stimulated AC activity in mice [56,57].

In addition to β-AR desensitisation and uncoupled AC activity, β-AR distribution patterns are also altered in HFrEF. A loss in T-tubular invaginations has been observed in many forms of human HFrEF (ischaemic, idiopathic-dilated and hypertrophic cardiomyopathy) and in pressure overload-induced animal...
models of HFrEF, with a loss in T-tubular structure detected early and for a worsening of T-tubular loss found to correlate with disease severity. For example, cardiomyocytes isolated from rats with abdominal aortic constriction-induced compensated LV hypertrophy, but with maintained basal and isoproterenol-stimulated cardiac performance, exhibited a ~14% loss in T-tubular density [58], while cardiomyocytes isolated from rats 16 weeks postmyocardial infarction (MI) exhibited LV hypertrophy and reduced ejection fraction (<50%), presented with a 50% loss in T-tubular density, similar to that observed in human HFrEF (ischaemic, idiopathic-dilated and hypertrophic cardiomyopathy) [59]. Further work combining FRET and SICM has allowed us to uncover that this loss in T-tubular structure is accompanied by a redistribution of β2-ARs from the T tubules to the cell crest of the cardiomyocyte, resulting in β2-AR-stimulated cAMP signals exhibiting similar propagation patterns to that of β1-AR signalling [39]. These data raise the question as to whether β2-AR redistribution in HFrEF alters the cardioprotective nature of β2-AR signalling. Indeed, β2-AR stimulation in failing human and mouse hearts has been reported to induce functional effect characteristic of β1-AR signalling [60]. Furthermore, it has been shown that reversal of heart failure in rats restores T-tubular structure and β2-AR distribution and cAMP propagation patterns [61], suggesting that maintaining normal β-AR distribution is essential in maintaining cardiac function.

These data suggest that in HFrEF, there is a desensitisation of the β1-AR and increased ionotropic actions of the β2-AR and that these changes correlate with disease severity and are mediated by remodelling of β-AR microdomains.

In future work, it would be interesting to determine the AC isoforms that the β2-AR couples with at the crest postredistribution. If there is still a bias towards β2-AR and AC5 coupling as seen in the T tubules, or a newly formed coupling between the β2-AR and AC6? Particularly as AC6 activity has been reported to be cardioprotective, understanding whether β2-AR redistribution aids in maintaining AC6 activity at the crest may give insight into mechanisms of remodelling during HFrEF that attempt to compensate for reduced β1-AR expression.

Beta-adrenergic receptor microdomains in HFP EF

There is evidence for disruptions in β-AR microdomains in HFP EF, with the obese/T2D heart exhibiting altered β-AR expression levels, blunted β-AR responsiveness and evidence of altered β2-AR-coupled PDE activity (Fig. 3).

Previous studies in animals suggest that β-AR signalling is impaired in obesity and T2D due to reduced expression of β-ARs, altered expression ratios of the β-ARs and/or internalisation of β-ARs. [62]. These effects appear to be dependent on the model and severity of disease. For example, human atrial tissue from diabetic patients exhibited decreased expression of both the β1-AR and β2-AR [63]. Right atrial strips from 14-week streptozotocin (STZ) diabetic rats exhibit a blunted response to β1-AR but not β2-AR stimulation. And in the hearts of STZ rats, the expression of the β1-AR at mRNA and protein is reduced, while β2-AR expression levels are decreased at the protein level and increased at the mRNA level [62]. In T2D diabetic fatty rats, time-dependent changes in protein expression of β-AR were observed, with no change in the protein levels of the β1-AR at 10 weeks but a reduction in β2-AR protein levels, while at 16 weeks a decreased expression of both the β1-AR and β2-AR proteins was observed [64]. A limitation in applying these data to HFP EF is that even though these models are readily applied to HFP EF research, diastolic function was not assessed in each of these studies. Therefore, it cannot be concluded at what stage alterations in β-AR expression may appear in HFP EF or if they do at all. However, a further rational for investigating the presence of impaired β-AR signalling in HFP EF is the presence of reduced cardiac reserve and exercise intolerance in HFP EF patients, with depressed myocardial oxygen supply observed in HFP EF particularly during exercise [65,66]. In addition, HFP EF patients have also been shown to exhibit a blunted response to dobutamine infusion, with dobutamine inducing a change in EF of just ~0.4% in HFP EF patients compared with 19% in control patients [67], suggesting the presence of β-AR desensitisation. However, it should be noted that the increased LV filling pressures present in HFP EF may also result in reduced cardiac reserve, and this is particularly apparent during exercise in which there is a further increase in LV filling pressures, for which the HFP EF heart has an inadequate ability compensated for with enhanced relaxation [66].

Considering the mechanisms for β-AR desensitisation in obesity/T2D, there is growing evidence to support a counter-regulation between insulin and β-AR signalling in the heart [67]. A defining characteristic of the obese and T2D heart is the development of cardiac insulin resistance [68]. Interestingly, sustained cardiac β-AR stimulation has been shown to promote cardiac insulin resistance and vice versa, and insulin
resistance has been shown to promote cardiac β-AR desensitisation. Under normal conditions, insulin promotes cardiac glucose uptake through the activation of the insulin receptor (Ins-R)-mediated phosphatidylinositol 3-kinase (PI3K)-Akt pathway, which induces glucose transporter 4 (GLUT4) translocation from internal stores to the sarcolemma increasing cardiac glucose uptake. Acute β-AR stimulation increases glucose uptake via PKA-mediated phosphorylation of Akt, while chronic β-AR stimulation reduces glucose uptake and causes insulin resistance due to sustained Akt activation and reduced translocation of GLUT4 [69,70].

A novel complex between the β2-AR and Ins-R has been identified, in which the Ins-R and β2-AR were suggested to form a complex at the membrane in mouse hearts [71]. In these studies, chronic insulin stimulation (in response to a HFD in vivo or insulin treatment in vitro) was shown to promote β-AR desensitisation via PKA- and GRK-mediated phosphorylation of the β2-AR and increased Gαi coupling, with cardiomyocytes from HFD mice exhibiting blunted contractile responses to isoproterenol stimulation and a reduced PKA-mediated phosphorylation of PLN. While it is not clear from these studies whether β1-AR signalling or phosphorylation was altered, inhibition of Gαi with pertussis toxin increased the isoproterenol-induced PKA phosphorylation of PLN in HFD cardiomyocytes, suggesting the alterations observed in HFD cardiomyocytes are at least in part mediated via β2-AR and Gαi coupling. Indeed, β2-AR KO mice fed a HFD exhibited normal cardiac reserve in response to β-adrenergic stimulation [71].

Supporting these findings, there is growing evidence that GRK2 plays a pivotal role in linking cardiac insulin resistance and β-AR desensitisation, as GRK2 is upregulated by both catecholamines and hyperinsulinaemia, with GRK2 expression increased not only during HFpEF but also in insulin resistance states such as in ob/ob mice or animals fed with high-fat diet [72]. Furthermore, cardiac-specific GRK2 KO mice show enhanced heart glucose metabolism [73]. GRK2 acts as a negative regulator of both insulin and β-AR signalling, with the ability to phosphorylate and inhibit not only β-ARs but also the Ins-R. Indeed, increased GRK2 levels have been reported to worsen glucose uptake after ischaemic injury via insulin receptor substrate 1 (IRS-1) phosphorylation [73]. Therefore, negative feedback signalling mediated via GRK2 is exacerbated by both chronic catecholamine and insulin signalling.

The first insights into microdomain-specific PDE activity in obesity and T2D have come from studies in a mouse line harbouring the cAMP biosensor, which is localised to the cytosol [71]. HFD-induced obesity and hyperinsulinaemia in these mice blunted β2-AR-stimulated cAMP production. These effects were hypothesised to be mediated via insulin and increased PDE4 activity, with PDE4 inhibition by rolipram-restoring β2-AR FRET signals to that of control mice. Western blot analysis identified increased PDE4D expression and no change in PDE4B, suggesting PDE4 isoform-specific regulation. These effects could be replicated in chronically insulin-treated control cardiomyocytes and were accompanied in both models by increased β2-AR phosphorylation at both PKA and GRK2...
phosphorylation sites. The authors hypothesised these findings explain the blunted systolic cardiac reserve observed in T2D. At this time, it is not clear how these findings relate to diastolic function [71,74]. It is also important to consider that while these studies were performed in a model of obesity and hyperinsulinaemia, which provided a nice phenotype for early, pre-T2D disease progression, these mice were not T2D and it is unlikely that at this stage the hearts exhibited insulin resistance. As such, future work elucidating how the proposed interaction between the Ins-R and β2-AR is affected in a model of overt T2D and in response cardiac insulin-resistant state is needed in order to relate these findings to obesity/T2D HFpEF.

In addition, it is important to note that while this review discusses cardiac insulin resistance in the context of HFpEF, cardiac insulin resistance is not unique to HFpEF. Indeed, HFrEF is also associated with the development of cardiac insulin resistance, with impaired insulin signalling and glucose uptake shown to precede or occur in response to pressure overload-induced HFrEF in the absence of metabolic diseases [69,73,75–78].

Despite the presence of chronic sympathetic activity in obesity and T2D-associated HFpEF, our understanding of β-AR expression and coupled cAMP signalling lags behind advances made in HFrEF. In the future, it would be exciting to employ the same advances in methodology that have been applied in HFrEF, such as SICM and FRET, to identify β-AR distribution patterns and diffusion patterns of cAMP signals. Particularly, the investigation into these alterations at the stage of early impaired LV filling to that in late-stage HFpEF. In addition, a comprehensive investigation into the expression of β-ARs (including phosphorylation and internalisation status), G protein subunits and AC isoforms with the parallel assessment of diastolic function in both human obesity/T2D HFpEF and appropriate animal models is needed. This work will allow us to identify how remodelling of β-AR microdomains coincides with the progression of obesity/T2D HFpEF.

**Calcium-handling microdomains**

For our heart to beat, an action potential-induced depolarisation opens the voltage-gated LTCCs allowing Ca$^{2+}$ to enter the cardiomyocyte. This increase in intracellular Ca$^{2+}$ triggers the opening of the RyRs allowing a larger amount of Ca$^{2+}$ release from the SR and initiating contraction. Relaxation is facilitated via Ca$^{2+}$ reuptake from the cytoplasm back into the SR, which is mediated by PLN-regulated SERCA2a.

**LTCC microdomains in the healthy heart**

LTCC is localised predominately to T-tubular invaginations on the sarcolemma [79]. These T-tubular invaginations are rich in caveolin, are 150–300 nm wide and are situated at the Z-line (the junction of each sarcomere). The LTCC is phosphorylated via PKA; in a healthy heart, β-AR/cAMP-dependent regulation of the LTCC microdomain has initially been proposed to be mediated via AKAP18z, which is a short isoform of AKAP7 [80] or by AKAP5 (also known as AKAP150/79) [81] and PDE4B (Fig. 1). AKAP5 was found to facilitate the formation of a complex containing AC5, PKA and calcineurin with the LTCC in mice. In AKAP5 knockout cardiomyocytes, calcium transients postisoproterenol stimulation were impaired, with a loss in PKA-mediated RyR phosphorylation and SR Ca$^{2+}$ release, despite isoproterenol inducing similar increases in cAMP production in AKAP5 KO and WT cardiomyocytes [81], highlighting the importance of AKAP5 localisation within the LTCC microdomain. While AKAP18z was found to co-immunoprecipitate with CaV1.2 channels in membrane fractions from the rat heart and to co-localise with CaV1.2 channels and PKA in the T tubules of rat ventricular cardiomyocytes [82], the knockout of AKAP7 had no effect on calcium cycling [83], suggesting that some other AKAPs may be involved in the regulation of the LTCC microdomain. The L-type Ca$^{2+}$ current ($I_{Ca,L}$) induced via β1-AR stimulation has been shown to be mediated via AC6. While the β2-AR has been shown to couple with the LTCC in frog cardiomyocytes and for β2-AR stimulation to induce LTCC phosphorylation in canine cardiomyocytes, it has also been shown in mouse cardiomyocytes that β2-AR stimulation fails to induce an $I_{Ca,L}$ due to PDE activity masking the actions of β2-AR-stimulated cAMP pools, with an $I_{Ca,L}$ postselective β2-AR stimulation only evident post-PDE3 and post-PDE4 inhibition. However, in contrast to this study observing PDE3 activity to regulate cAMP pools within the LTCC, co-immunoprecipitation studies have only pulled down PDE4B and PDE4D with the pore-forming subunit CaV1.2 of the LTCC in mouse hearts. PDE4B was found to limit the $I_{Ca,L}$, thus preventing calcium overload via LTCC stimulation. Furthermore, disruption of PDE4B activity in PDE4B −/− mice leads to increased β-AR-induced cell contraction and Ca$^{2+}$ transients, while no effect was observed in PDE4D −/− mice. However, again in contrast to this, other studies have observed both PDE4B and PDE4D to regulate $I_{Ca,L}$ in response to β-AR stimulation [84].
Basal activity of PDEs within the LTCC is not suggested to regulate LTCC activity, as in rat cardiomyocyte basal inhibition of PDE2, PDE3 and PDE4 did not increase LTCC phosphorylation [85].

The generation of a cAMP biosensor targeted to the caveolin-rich microdomains (pmEpac1-camps) has provided key insights into real-time PDE activities near the LTCC. In this work, cardiomyocytes from a mouse line expressing the Epac1-camps biosensor were selectively stimulated via the β1-AR or β2-AR and associated PDE activities were assessed. This work identified β-AR subtype-specific differential PDE regulation of cAMP at the LTCC/plasma membrane microdomain, with β1-AR-induced cAMP regulated via PDE4, PDE3 and PDE2 and β2-AR-stimulated cAMP regulated predominantly via PDE3 followed by PDE4 [86].

**LTCC microdomains in HFrEF**

cAMP dynamics within the LTCC microdomains are altered in HFrEF due a redistribution of LTCC to the crest and an associated uncoupling with the RyR, and a redistribution of PDE2 activity between the β1-AR and β2-AR (Fig. 2).

Coupling between the LTCC and RyR is disrupted in HFrEF in part due to the decreased T-tubular invaginations observed in many forms of human HFrEF (ischaemic, idiopathic-dilated and hypertrophic cardiomyopathy) [59,87] and in numerous pressure overload-induced animal models of HFrEF [88]. As mentioned, studies in ventricular cardiomyocytes isolated from rats 4, 8 and 16 weeks post-MI identified a worsening of T-tubular loss correlating with disease severity [89]. This loss in T-tubular structure leaves the RyRs ‘orphaned’ on the SR, preventing the formation of dyads and thus amplification of the ICa,L, resulting in a decreased RyRr opening in heart failure. In addition, a redistribution of both LTCC and RyR has also been observed in heart failure, with a redistribution of LTCC to the crest of sarcolemma in human hearts from patients with dilated cardiomyopathy and in rat cardiomyocytes 16 weeks post-MI [88] and an increased density of RyR at the ends of the T-tubular invaginations.

PDE2 expression and activity are increased by two-fold in the ventricular tissue from end-stage heart failure patients (with dilated or ischaemic cardiomyopathy) compared with that in donor hearts. And in rats, catecholamine stimulation increases both the expression and activity of PDE2 by two- and four-fold, respectively, thereby blunting β-AR responsiveness. Considering how these data relate to the LTCC microdomain, the overexpression of PDE2 reduced Ca2+ entry through the LTCC preventing an inotropic response via β-AR stimulation [90]. These findings have been supported by FRET studies assessing cAMP dynamics within the LTCC microdomain using the pmEpac1-camps biosensor [91], in which FVB/N1 mice subjected to TAC exhibiting a mild compensated HF phenotype showed increased isoproterenol-stimulated cAMP production via the β1-AR and decreased cAMP production via the β2-AR, accompanied by a redistribution of PDE2 activity between the β1-AR and β2-ARs with less PDE2 activity regulating cAMP pools generated via β1-AR stimulation and more PDE activity via β2-AR stimulation. Interestingly, these hearts exhibited no changes in PDE activity or expression at the whole-cell level, suggesting mild heart failure is accompanied by alterations in PDE activity and distribution at the microdomain level that precede whole-cell changes in PDE activity and expression, which have been reported in late-stage heart failure.

In future work, it would be interesting to assess HFrEF promoted changes in PDE activities within the LTCC microdomain with the ability to stimulate an ICa,L via the β2-AR. As a decreased PDE3 regulation of β2-AR-cAMP pools was identified post-TAC in mice and as PDE3 and PDE4 activities have previously been shown to prevent β2-AR-stimulated ICa,L, it would be interesting to determine whether the blunted PDE3 effects observed post-TAC contribute to an increased inotropic effect of the β2-AR at the LTCC. In addition, it will be exciting to elucidate differences between early- and late-stage alterations in PDE activities within the LTCC microdomain, in particular to assess whether cAMP stimulation via the β1-AR remains increased or whether there is blunted β1-AR-cAMP signalling within the LTCC at late-stage HFrEF.

**LTCC microdomains in HFpEF**

There is evidence to support a remodelling of the LTCC microdomain in HFpEF, with obese/T2D hearts exhibiting disruptions in T-tubular structure and an uncoupling between the LTCC and RyR (Fig. 3).

Considering T-tubular structure, previous studies support a disruption in T-tubular structure in obesity/T2D HFpEF as seen in HFrEF. db/db mice have been shown to exhibit ~40% reduced T-tubular density that was associated with increased diastolic SR Ca2+ leak [92]. Likewise, in a rat model of T2D, a pronounced decline in functionally intact SR/T-tubular junctions and a displacement of transverse T tubules in a longitudinal direction were observed [93].
However, another study reported no T-tubular remodelling in STZ diabetic rats, with no change in the spatial distance between T tubules and SR membranes detected [94]. Interestingly, exercise in db/db mice, which is supposed to improve systolic and diastolic function, also increased T-tubular density [92], further supporting the importance for normal T-tubular structure in maintaining cardiac performance. However, it is not clear whether T-tubular structure is altered in human obese/T2D cardiomyocytes.

Considering how this loss in T-tubular structure may impact insulin and β-AR signalling, GLUT4 translocation has been shown to occur in both the T tubule and crest of the cardiomyocyte [95], with insulin-stimulated GLUT4 translocation shown to predominately occur in the T tubule over the crest [96]. Furthermore, dissociation of T tubules has been shown to reduce basal and abolish insulin-dependent glucose uptake in skeletal muscle [97,98]. Therefore, the loss of T-tubular structure in the obese/T2D cardiomyocyte may have significant impacts not only on β-AR signalling but also on substrate handling, exacerbating the diminished capacity for insulin-stimulated glucose uptake already present. It is unclear how these alterations may impact the proposed interaction between the Ins-R and β2-AR.

Previous studies also support impaired LTCC activity and LTCC and RyR coupling in models used for HFpEF research. A reduction in PI3K-Akt signalling has been shown to disrupt the function of the LTCC in db/db cardiomyocytes, with db/db cardiomyocytes exhibiting a reduction in ICa,L that could be restored to normal levels with infusion of phosphatidylinositol 3,4,5 trisphosphate (the second messenger produced by PI3K) [99], suggesting insulin resistance impairs the LTCC. This is supported by studies assessing the ICa,L in response to energy deprivation induced via carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP), which uncouples mitochondrial oxidative phosphorylation, thus reducing ATP production (a phenotype characteristic to cardiac insulin resistance in which mitochondrial oxidation and myocardial ATP content are reduced [68]). FCCP treatment has been shown to cause an acute transient increase in ICa,L amplitude that is quickly followed by a reduction, with the initial increase possibly due to acute reduction of Ca2+ release from SR resulting in reduced calcium-dependent inactivation of the LTCC [100].

Interestingly, in contrast to end-stage HFrEF, PDE2 expression is not changed in LV tissue from patients with hypertrophy and preserved cardiac function (EF % > 50%) undergoing an aortic valve replacement. While aortic stenosis is commonly accompanied by diastolic dysfunction [101], it is not clear whether these patients exhibited diastolic dysfunction or whether they were obese and/or T2D. Furthermore, while no change in PDE2 expression was detected in tissue homogenates from these patients, it is unknown whether PDE2 activity specifically within the LTCC was also unchanged. Indeed, as mentioned, alterations in PDE activity or localisation have been reported to occur prior to whole-cell changes in PDE activity and expression in HFrEF. It is not known whether the same phenomenon occurs in HFpEF.

In future work, it would be interesting to gain a greater understanding of T-tubular loss and displacement as seen in past studies in db/db and T2D rat cardiomyocytes and to study whether a loss in T-tubular density precedes the development of HFpEF and whether it is accompanied by a redistribution of the β2-AR to the crest as seen in HFrEF. Furthermore, it is unclear whether a loss in T-tubular structure impacts the formation of the proposed β2-AR and Ins-R complex. It would also be exciting to assess PDE activity in living cardiomyocytes expressing a cAMP biosensor in close proximity to the LTCC (such as the pmEpac1-camps biosensor), to determine whether a change in PDE activity within the LTCC is responsible for the blunted ICa,L observed in the obese/T2D cardiomyocyte, such as an increase in PDE2 activity as seen in HFrEF. Also, it would be exciting to determine whether there are any differences in β-AR subtype regulation of cAMP within the LTCC.

**RyR microdomains in the healthy heart**

The RyR is a tetramer comprised of four type 2 RyR subunits (RyR2) and four FK506-binding proteins (FKBP12.6). During diastole, RyR channels are closed, and PKA phosphorylation of RyR2 dissociates FKBP12.6 opening the RyR channel. The LTCC is within close proximity to the RyR on the sarcoplasmic reticulum (SR), ~12–15 nm apart [59]. This area between the LTCC and RyR forms a cardiac dyad, in which small amounts of Ca2+ entry from the ICa,L can be confined to a smaller region. cAMP regulation within the RyR microdomain has been proposed to be mediated via muscle selective A-kinase anchoring protein (mAKAP) and PDE4D3 [102,103] (Fig. 1) with mAKAP and PDE4D3 co-immunoprecipitating with the RyR in rat and human hearts [102]. In rat cardiomyocytes, disruption of the PKA and mAKAP interaction blunts stimulated PDE4D3 activity, suggesting the mAKAP complex is required for negative feedback signalling [103]. In Pde4d −/− mice, the
RyR2 is hyperphosphorylated by PKA, resulting in abnormal ‘leaky’ SR Ca\(^{2+}\) release, increased sensitivity to arrhythmias, development of dilated cardiomyopathy and accelerated progression of myocardial infarction-induced heart failure [104]. However, it should be noted that other studies have challenged these past findings, with conflicting data suggesting that mAKAP is not localized to the SR and instead situated at the nuclear envelope [105,106]. Future work is needed to confidently assess which AKAP(s) does indeed associate with the RyR.

In rat cardiomyocytes, basal phosphorylation of RyR could not be achieved with inhibition of PDE2, PDE3 or PDE4, suggesting PDEs do not act to limit cAMP activity under basal conditions within the RyR microdomain [85]. This is in contrast to the hypothesis by Dodge et al (2001) that under basal conditions, mAKAP tethering of PDE4D3 prevents basal activation of PKA. In order to gain greater insights into real-time cAMP dynamics in the RyR microdomain, a FRET biosensor was developed targeted to the RyR (Epac1-JNC). In healthy cardiomyocytes expressing the Epac1-JNC biosensor, selective β₁-AR but not β₂-AR stimulation strongly increased cAMP levels within the RyR2 microdomain. These β₁-AR-cAMP pools were regulated predominately via PDE4 followed by a smaller contribution by PDE3, with little to no PDE2 regulation. Interestingly, these PDE4 effects in the RyR microdomain were two- to threefold greater than that in the cytosol, consistent with past evidence that PDE4 regulates cAMP dynamics within the RyR microdomain [107].

**RyR microdomains in HFrEF**

cAMP dynamics within the RyR microdomains are altered in HFrEF due to increased β₂-AR-cAMP signalling, hyperphosphorylation of the RyR by PKA and decreased local PDE4 and increased PDE2 and PDE3 activities (Fig. 2).

Consistent with observations in PDE4D −/− mice, in HFrEF there is also a decreased PDE4D activity and increased PKA-mediated hyperphosphorylation of the RyR, with human failing heart tissue exhibiting a decreased binding of the FKBP12.6 regulatory subunit and decreased association with PDE4D3, resulting in impaired function and increased sensitivity to Ca\(^{2+}\)-induced activation [102]. These data are further supported by studies in mice identifying PDE4D3 to be part of the RyR2 complex, with PDE4D3 association with RyR reduced in response to heart failure. They also suggest that PDE4D deficiency may contribute to heart failure by chronic RyR hyperphosphorylation.

There are some conflicting data, since this study has observed that RyR2-associated PDE activity was almost completely inhibited by rolipram but not by milrinone, suggesting PDE3 does not regulate cAMP activity within the RyR microdomain. However, in mouse cardiomyocytes both PDE3 and PDE4 have been shown to be required to maintain basal cAMP-PKA signalling within the RyR2 microdomain [108]. Furthermore, while FRET studies using Epac1-JNC biosensor revealed PDE4 to be the main PDE family responsible for regulating isoproterenol-induced cAMP pools within the RyR microdomain, PDE3 inhibition was also able to increase cAMP levels at the RyR microdomain [107].

To study cAMP dynamics in the RyR microdomain in the setting of HFrEF, FVB/N1 mice expressing the Epac1-JNC biosensor were subjected to TAC, inducing a compensated cardiac hypertrophy and early HF phenotype. Cardiomyocytes isolated from these mice exhibited a redistribution of PDE activity and β₂-AR subtype signalling [107], with increased contributions of PDE2 and PDE3 in regulating isoproterenol-induced cAMP and a decreased contribution of PDE4, consistent with past reports of decreased PDE4D3 association with the RyR. These cardiomyocytes also exhibited a dramatic increase in β₂-AR-stimulated cAMP levels within the RyR microdomain, which was not observed in healthy cardiomyocytes. These alterations were associated with increased SR Ca\(^{2+}\) leak, suggesting impaired RyR activity and also suggest that the increased activities of PDE2 and PDE3 are insufficient to compensate for reduced PDE4 activity within the RyRmicrodomain.

In the future, it would be interesting to investigate how the newly developed reliance on β₂-AR within the RyR microdomain evolves in response to a more severe HFrEF phenotype. Furthermore, the mechanisms responsible for reduced PDE4 activity within the RyR are also unclear.

**RyR microdomains in HFpEF**

There is evidence to suggest a disruption in RyR microdomains in HFpEF, with impaired LTCC/RyR coupling and SR Ca\(^{2+}\) leak observed in obesity/T2D. However, the mechanisms behind this are unclear (Fig. 3), with inconsistent reports regarding RyR expression and phosphorylation state. For example, in human T2D atrial tissue mRNA expression and protein expression of the RyR were reduced by 68 and 22%, respectively (these patients showed no differences in PLN or SERCA2a expression). 15-week-old db/db obese and T2D hearts exhibit impaired LTCC/RyR
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coupling with a decrease in RyR density and decreased SR Ca\(^{2+}\) release, which was hypothesised to be due to a blunted \(I_{Ca,L}\) to stimulate RyR SR Ca\(^{2+}\) release and due to reduced LTCC expression [109]. In contrast, another study in 12-week-old \(db/db\) mice reported no change in RyR expression but a reduction in the expression of FKBP 12.6, which may have promoted the increased SR Ca\(^{2+}\) leak in the \(db/db\) cardiomyocytes [110]. However, other studies suggest the increased SR Ca\(^{2+}\) leak in obesity/T2D is mediated via hyperphosphorylation of the RyR [111]. In HFD-fed dogs, the RyR was hyperphosphorylated at the PKA site Ser2809 [112] and STZ rats were found to be exhibit both RyR hyperphosphorylation at the PKA site and reduced protein expression of the RyR and FKBP12.6.

In future work, it would be interesting to investigate PDE coupling with the RyR, and this may assist in gaining some insight into the mechanisms promoting the presence of the SR Ca\(^{2+}\) leak in obesity/T2D. For example, it would be interesting to investigate whether RyR coupling with PDE4 is reduced in HFrEF as it is in HFrEF. This may provide an explanation for RyR hyperphosphorylation. In addition, the application of FRET cAMP biosensors such as the Epac-JNC will be integral to gain insight into alterations in PDE activity within the RyR microdomain and \(\beta\)-AR subtype signalling, to determine whether \(\beta_2\)-AR reliance is increased in HFrEF as it is in early-stage HFrEF.

**PLN/SERCA2a microdomains in the healthy heart**

PLN binds to and decreases the affinity of SERCA2a for Ca\(^{2+}\). The phosphorylation of PLN by PKA results in the dissociation of PLN from SERCA2a, allowing diastolic SR Ca\(^{2+}\) reuptake. PLN has been shown to form a complex with AKAP18\(\delta\) and PDE4D or PDE3A (Fig. 1), with AKAP18\(\delta\) acting as a scaffold protein that coordinates PKA phosphorylation of PLN. AKAP18\(\delta\) immunofluorescence exhibits a striated pattern overlapping with that of PLN and SERCA2 in rat cardiomyocytes, and cAMP pull-down using sarcoplasmic reticulum fractions and a AKAP18\(\delta\) antibody yielded PKA-RII\(\alpha\), PLN and SERCA2 [113]. Disruption of AKAP18\(\delta\) tethering of PKA in rat cardiomyocytes impairs PLN phosphorylation, preventing the dissociation of PLN from SERCA2, thereby attenuating Ca\(^{2+}\) reuptake and relaxation [113], suggesting an integral role for the AKAP18\(\delta\) complex within the PLN/SERCA2a microdomain in facilitating PKA-mediated phosphorylation of PLN. Considering PDE activity, co-immunoprecipitation experiments have shown PDE4D to co-immunoprecipitate with SERCA2a in both human and mouse hearts [114]. The presence of PDE4 in PLN/SERCA microdomain is supported by immunofluorescence identifying PDE4 subcellular localisation to the Z-band of cardiomyocytes. In mice, PDE3A has been found to co-immunoprecipitate with both SERCA2a, PLN, AKAP18\(\delta\) and protein kinase type A-RII and for basal PDE3 inhibition to increase PLN phosphorylation [115].

Fusion of the Epac1-camps biosensor with SERCA2a (Epac1-camps-PLN) allowed for investigation of cAMP dynamics within the PLN/SERCA2a microdomain [116]. Strong \(\beta_1\)-AR but not \(\beta_2\)-AR-cAMP signals were present in the SERCA2a microdomain, consistent with previous studies showing no effect of \(\beta_2\)-AR-cAMP signals on PLN phosphorylation [22]. Interestingly when comparing cAMP responses within the SERCA2a microdomain compared with those obtained in the bulk cytosol (Epac1-camps biosensor), the SERCA2a microdomain exhibited fourfold higher stimulated cAMP levels than that in the cytosol. PDE inhibition with the pan PDE inhibitor IBMX blunted these differences, suggesting that PDEs act to facilitate communication between the \(\beta_1\)-AR microdomain and SERCA2a microdomain. Assessment of the contribution of individual PDEs identified that PDE3 and PDE4 work together to regulate this cross-microdomain communication. Basal PDE3 and PDE4 activities were also seen to restrict cAMP within this microdomain to prevent PLN phosphorylation.

**PLN/SERCA2a microdomains in HFrEF**

cAMP dynamics within the PLN/SERCA2a microdomains are altered in HFrEF due to blunted \(\beta_1\)-AR-cAMP signals, reduced SERCA2a and basal PDE4 activity and increased PDE2 activity (Fig. 2).

In human end-stage heart failure (ischaemic, idiopathic-dilated and hypertrophic cardiomyopathy) and animal models of HFrEF, SERCA2a expression and activity are decreased [117–119]. Increasing expression of SERCA2a is able to improve cardiac function in human-advanced heart failure (EF% < 30 \%) and animal models of pressure overload-induced HFrEF, including in rats exposed to TAC pressure overload for 23 weeks during which they transited from compensated hypertrophy to heart failure [120–122].

In response to TAC induced hypertrophy and early heart failure (reduced systolic function but EF % still > 50 \%), cardiomyocytes no longer exhibited higher isoproteino-induced cAMP levels at the SERCA2a domain compared with that in the bulk cytosol [116], suggesting communication between the \(\beta_1\)-AR
microdomain and SERCA2a microdomain is impaired in response to TAC. While whole-cell PDE activities and expression were not altered, alterations in PDE activity at the microdomain level were observed, consistent with previous findings in early heart failure models, with the SERCA2a microdomain exhibiting a decreased basal PDE4 activity and increased isoproterenol-stimulated PDE2 activity, suggesting TAC induced alterations in PDE distribution may mediate the disruption between β-AR microdomain and SERCA2a microdomain communication.

In future work, it would be interesting to assess the PLN/SERCA2a microdomain in a setting of late-stage HFpEF and to gain insight into selective β-AR subtype stimulation on PDE activities and PLN phosphorylation.

**PLN/SERCA2a microdomains in HFpEF**

There is evidence to suggest a disruption in RyR microdomains in HFpEF with SERCA2a activity reported to be reduced in the obesity/T2D heart, with reduced mRNA and protein expression of SERCA2a and reduced PLN phosphorylation (Fig. 3).

For example, the T2D Otsuka Long Evans Tokushima fatty rat exhibited diastolic dysfunction and decreased SERCA2a expression [123]. db/db mice exhibited a marginal decrease in SERCA2a expression and a large increase in PLN expression with the PLN/SERCA2a ratio approximately threefold higher in db/db mice than in control. db/db mice also exhibited reduced PLN phosphorylation that may have contributed to an increased inhibitory effect of PLN on SERCA2a [110]. Sucrose-fed rats that exhibited hyperinsulinaemia, hyperglycaemia and hyperlipidaemia exhibited a marginal decrease in SERCA2a levels reported in Zucker diabetic fatty (ZDF) rats and in ZDF rats administered insulin [125], and this reduction in SERCA2a was accompanied by reduced PLN expression resulting in an increased SERCA/PLN ratio in both ZDF and ZDF + insulin rats and an associated increased SR Ca^{2+} uptake. This early T2D model is unlikely to present with overt cardiac insulin resistance, and as such, these data support the detrimental effects of impaired cardiac insulin signalling on PLN/SERCA2a function. Indeed, pretreatment of WT cardiomyocytes with insulin reduces isoproterenol-stimulated PKA phosphorylation of PLN [74].

In the future, it would be interesting to uncover how PDE activity and/or PKA localisation within the PLN/SERCA2a domain in HFpEF is altered, whether an increased PDE activity or alterations in AKAP tethering of PKA may be blunting cAMP-induced PKA activation and subsequent PKA-mediated phosphorylation of PLN. In addition, there are other microdomains that are relevant to diastolic function that would be essential to elucidate cAMP signalling in HFpEF, including the troponin I (TnI) microdomain for which there is already a developed FRET biosensor [126].

**Summary and outlook**

Over recent years, advances in technology have greatly aided in our understanding of cAMP microdomains in HFrEF. In particular, the generation of targeted FRET-based cAMP biosensors has allowed the intricate steps of cAMP stimulation and regulation within distinct microdomains to evolve from a theoretical concept to a phenomenon that we can not only visualise, but also manipulate and measure in real time, and have allowed us to shed light into the intricate remodelling of PDEs providing an explanation for altered activity of calcium-handling proteins during early stages of HFrEF in the absence of whole-cell alterations in PDE expression and activity. It is clear in HFrEF that there is a disruption in T-tubular structure and a desensitisation and redistribution of β-AR signalling that is accompanied by microdomain-specific alterations in PDE localisation and activity. Future work in understanding how these remodelling events differ in early stage and late stage of disease and in the different clinical presentations of HFrEF is essential in efforts to develop targeted intervention strategies.

Considering our understanding of cAMP microdomains in obesity/T2D HFpEF, there are many challenges we face, most notably the lack of readily available human HFpEF tissue for analysis due to limited cardiac transplantations in HFpEF and the difficulty in trying to rely on animal models to understand the pathophysiology of a disease driven by a multitude of diverse factors such as obesity and T2D, increasing age and hypertension/pressure overload stresses. Considering the high burden obesity and T2D place on the current and future prevalence of HFpEF, this review chose to focus specifically on cAMP microdomains related to these stresses. However, even by narrowing down the scope of the review we were still met with limitations when compiling the data from past literature. In particular, studies that jointly assessed diastolic function with cAMP dynamics in β-AR and
calcium-handling microdomains in the setting of obesity and T2D are mostly lacking. The majority of past literature has focused on systolic function, and our understanding of how alterations in cAMP microdomains contribute to diastolic dysfunction and vice versa remains largely unclear. In writing this review, we have, however, gained insight into the current stage of knowledge allowing us to identify major knowledge gaps and future work that is needed. Most notably, there is lack of data assessing cAMP dynamics in living cardiomyocytes at the microdomain level in obesity/T2D HFpEF. Studies in HFrEF have taught us that relying on whole-cell PDE activities and expression may hinder our ability to uncover early alterations in cAMP microdomain remodelling. As such, future work is needed to understand cAMP microdomains at the stage of impaired LV filling and late-stage HFpEF in obesity and T2D.

During this time, close attention can be paid to a few targeted strategies in cAMP signalling that have recently been tested or are currently being employed in clinical trials. For example, for the treatment of HFrEF, there are ongoing attempts to upregulate SERCA2a activity despite discouraging results of initial gene therapy trials [121,127]. There is also upcoming clinical work to test the disruption of SERCA2a/PDE3A interaction to locally increase cAMP and improve calcium cycling, and for HFpEF treatment, the use of the β3-AR agonist mirabegron (NCT02775539 and NCT02599480); istaroxime, the SERCA2a activator (NCT02772068, currently an inactive trial); and milrinone, the PDE3 inhibitor [128].

In a more general sense, it seems tempting to put forward a hypothesis that each form of HF, not only HFpEF and HFrEF but also their numerous aetiological entities, might have their own specific pattern of cAMP microdomain alterations, which should be better understood to allow more effective individualised new treatment options.

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Conflicts of interest
None to declare.

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