

LETTERS

EPAC regulation of cardiac EC coupling

We read with great interest the study by Pereira *et al.* (2007) and the accompanying Perspectives by Bers (2007) which were recently published in the *The Journal of Physiology*. These reports discuss a novel role for Epac, a cAMP-activated guanine nucleotide exchange factor, in the regulation of Ca²⁺ release during cardiac excitation–contraction (EC) coupling. The study of Pereira *et al.* found that electrically evoked Ca²⁺ release in adult rat cardiomyocytes was reduced following Epac activation by the selective activator 8-CPT. Moreover, this decrease in Ca²⁺ release during EC coupling was due to a Ca²⁺–calmodulin kinase II (CaMKII)-dependent reduction in sarcoplasmic reticulum (SR) Ca²⁺ load that results from an increased frequency of Ca²⁺ sparks leading to SR Ca²⁺ leak and store depletion. While these results implicate an exciting and novel role for Epac activation in the regulation of cardiac EC coupling, they diverge with prior published results from our laboratories showing clear Epac-mediated potentiation of Ca²⁺ release in adult mouse ventricular myocytes (Oestreich *et al.* 2007). Given the divergent conclusions of the two studies, we feel that it is important to reconcile these differences by providing a fair and comprehensive comparison of the experiments conducted in both reports. Additionally, such an examination might lead to a more comprehensive understanding of the potential physiological role of the Epac signalling pathway in cardiac myocyte function.

Oestreich *et al.* (2007) demonstrated that acute activation of Epac by 8-CPT increased electrically evoked Ca²⁺ release in single mouse ventricular myocytes, while 8-CPT had no effect on Ca²⁺ release in myocytes isolated from mice lacking phosphatidylinositol-specific phospholipase C ϵ (PLC ϵ). Moreover, adenoviral-mediated PLC ϵ expression in myocytes from PLC ϵ knockout mice restored 8-CPT enhancement of electrically evoked Ca²⁺ release. Thus, Epac-dependent regulation of electrically evoked Ca²⁺ release in the heart is entirely dependent on the presence of PLC ϵ . This is logical because previous work by us and others demonstrated that PLC ϵ enzymatic activity is directly regulated by Rap, making a direct connection between

Epac and PLC ϵ through Rap activation (Schmidt *et al.* 2001; Kelley *et al.* 2004). Importantly, inhibition of Rap activation with RapGAP significantly inhibited β -adrenergic receptor (AR)-dependent enhancement of Ca²⁺ release, placing this pathway downstream of β AR signalling. Consistent with a role for Epac and PLC ϵ in acute β AR-dependent enhancement of Ca²⁺-induced Ca²⁺ release (CICR), β -adrenergic-dependent stimulation of CICR was significantly reduced in myocytes isolated from PLC ϵ ^{-/-} mice. The relative role of the Epac and PKA pathways in β AR signalling was examined with specific inhibitors (H89, Rp-cAMPs) and activators (6-Bnz-cAMP) of PKA. Surprisingly, these experiments revealed that only 50–60% of β AR-dependent increase in evoked Ca²⁺ release could be attributed to PKA, with the remainder dependent on the novel Epac–Rap–PLC ϵ pathway (Oestreich *et al.* 2007). This conflicts with some reports where H89 completely inhibits β AR-dependent increases in CICR (Curran *et al.* 2007). A potential explanation for this difference is that all of our experiments were performed during strong stimulation of the β AR pathway (1 μ M isoproterenol (ISO; isoprenaline)). Thus, it is possible that the PKA-independent Epac pathway only operates during strong β AR signalling, consistent with the lower affinity of cAMP for Epac relative to PKA. A still unanswered question in these studies is the ultimate mechanism for how PLC ϵ activity alters Ca²⁺ release during EC coupling.

The recent work by Pereira *et al.* potentially sheds new insight on this question. This work shows that activation of Epac by 8-CPT leads to CaMKII phosphorylation at the auto-phosphorylation site and subsequent CaMKII-dependent phosphorylation of the ryanodine receptor (RyR2). Epac activation also increased Ca²⁺ spark frequency in a CaMKII-dependent manner. Since an increase in spark frequency reflects a higher sensitivity of RyR2 to activation by basal Ca²⁺ levels, this effect could account for the enhanced electrically evoked Ca²⁺ release observed in our prior study (Oestreich *et al.* 2007). In fact, CaMKII activation has been shown to contribute to ISO-dependent increases in CICR in the heart (Wang *et al.* 2004; Maier *et al.* 2007; Curran *et al.* 2007). How Epac activates CaMKII is not clear from the Pereira

et al. study. Consideration of our previous work demonstrating PLC ϵ dependence of the acute effects of Epac activation on cardiac Ca²⁺ handling suggests that the missing link between Epac activation and CaMKII activation may involve products of PLC ϵ activity. PLC ϵ hydrolyses PIP₂ to produce IP₃ and DAG leading to subsequent IP₃ receptor-dependent Ca²⁺ release or PKC activation, respectively. Interestingly, O-Uchi *et al.* (2005) found that α_1 -adrenoceptor stimulation in the heart leads to CaMKII activation in a PKC-dependent manner. However, further work will be required to determine if the Epac activation of CaMKII observed in the experiments of Pereira *et al.* results from an Epac–PLC ϵ –PKC pathway. Additionally, PLC ϵ is a unique PLC isoform in that in addition to its traditional catalytic function it also contains a guanine nucleotide exchange factor domain that functions as an activator of Rap. Clearly, delineating the relative role of the different PLC ϵ -dependent signalling elements in CaMKII activation during β AR stimulation will require further investigation.

A caveat of this discussion is that the observed effect of Epac activation on Ca²⁺ release during cardiac EC coupling are diametrically opposed between the studies of Oestreich *et al.* (2007) and Pereira *et al.* (2007) (see Fig. 1). Specifically, we found that acute treatment with 8-CPT increased electrically evoked Ca²⁺ release whereas the work by Pereira *et al.* showed a reduction. The reason for these disparate results are not completely clear, but one possible explanation is that the experiments conducted by Oestreich *et al.* involved acute exposure to 8-CPT for only 20 s prior to 60 s of 1 Hz electrical stimulation. Significant SR Ca²⁺ depletion may not occur during this brief exposure period, and in fact, we have observed that caffeine-induced responses were not different between control and 8-CPT-treated myocytes (authors' unpublished results). On the other hand, Pereira *et al.* found that significant SR Ca²⁺ depletion was observed using a longer (5 min) treatment protocol. However, it is important to consider that β AR stimulation of the Epac pathway occurs in parallel with PKA stimulation, which would limit SR Ca²⁺ depletion through PKA effects on phospholamban phosphorylation and SERCA-mediated SR

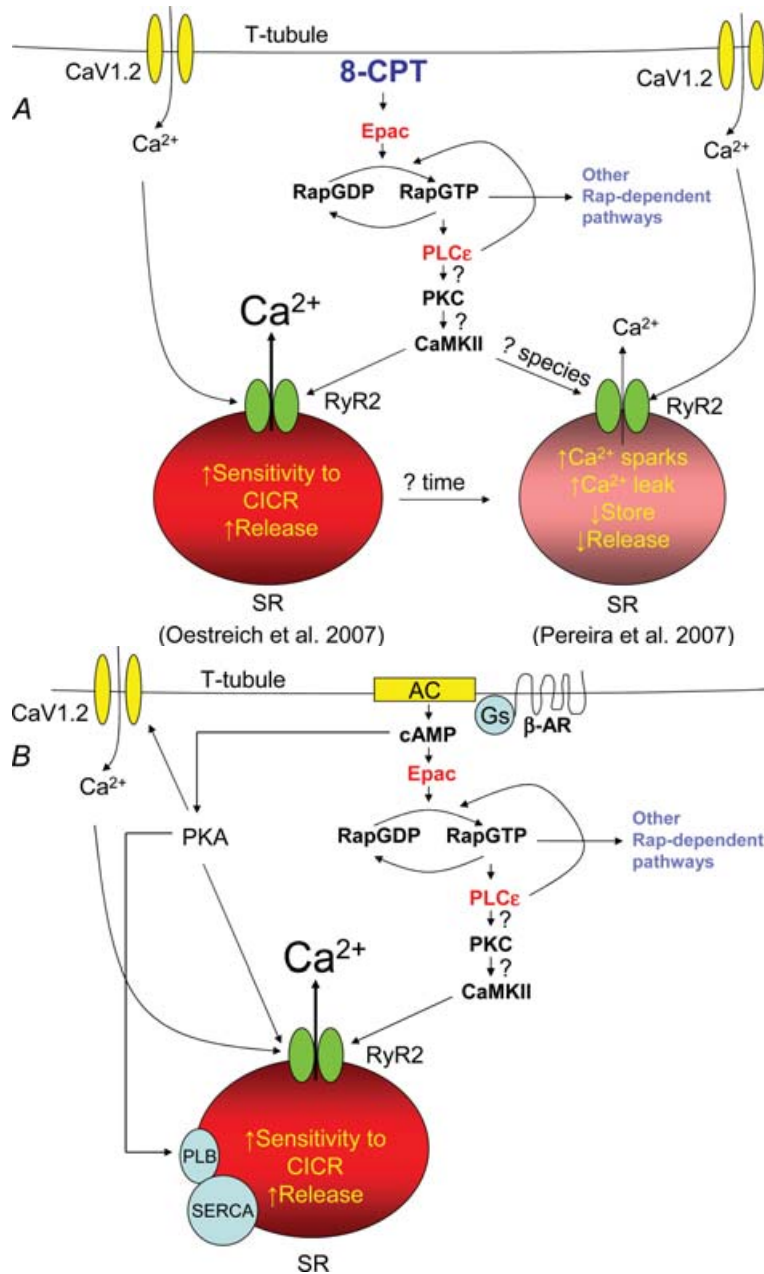


Figure 1. Roles for the Epac-PLC ϵ pathway in cardiac calcium release

A, treatment of isolated cardiac myocytes with 10 μM 8-CPT activates Epac and PLC ϵ , ultimately leading to CaMKII-dependent phosphorylation of RyR2 and either enhancement (Oestreich *et al.* 2007) or inhibition (Pereira *et al.* 2007) of Ca²⁺-induced Ca²⁺ release (CICR). PLC ϵ Rap guanine nucleotide exchange factors (GEF) activity is also shown to potentiate Rap activation and trigger additional downstream signalling pathways that may impact susceptibility to hypertrophy. **B**, Oestreich *et al.* provide evidence that activation of the Epac-Rap-PLC ϵ pathway occurs in parallel with PKA activation under conditions of strong β -AR stimulation. Thus, during concerted PKA and Epac activation, RyR2 activity is shown to be influenced by both PKA and CaMKII phosphorylation and SR depletion prevented by PKA-dependent SERCA activation.

Ca²⁺ reuptake. Alternatively, differences between the two studies might also reflect inherent species differences between rat and mouse myocytes.

Complete resolution of the physiological impact of Epac activation on Ca²⁺ dynamics during EC coupling requires further analyses. Additional insight into the physiological role of this novel pathway could be gleaned from the cardiac phenotype of PLC ϵ ^{-/-} mice (Wang *et al.* 2005). Since the Epac-dependent Ca²⁺ release pathway is completely dependent on PLC ϵ (Oestreich

et al. 2007), these mice lack Epac signalling in the heart. Isoproterenol-dependent enhancement of left ventricular developed pressure is significantly reduced in PLC ϵ ^{-/-} mice, consistent with the Epac-Rap-PLC ϵ pathway being regulated by β AR-dependent activation of Epac through cAMP (Wang *et al.* 2005; Oestreich *et al.* 2007). Surprisingly, PLC ϵ ^{-/-} mice are more sensitive to the development of hypertrophy. This result is inconsistent with the putative prohypertrophic role of CaMKII (Maier & Bers, 2007; Anderson, 2007) and the

previously reported prohypertrophic effects of Epac activation (Morel *et al.* 2005), but is consistent with a protective role for Epac activation described by Dodge-Kafka *et al.* (2005). One possibility is that Epac activation of PLC ϵ may activate additional cardioprotective pathways that balance the prohypertrophic effects of CaMKII activation. Overall, the recent studies of Epac regulation of cardiac Ca²⁺ regulation (Wang *et al.* 2005; Oestreich *et al.* 2007; Pereira *et al.* (2007) provide exciting evidence for a novel signalling mechanism

for the control of cardiac Ca²⁺ dynamics during EC coupling in health and disease.

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