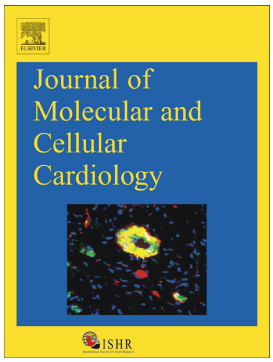

Dual mode of action of IP3-dependent SR-Ca2+ release on local and global SR-Ca2+ release in ventricular cardiomyocytes

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Dual Mode of Action of IP_3-Dependent SR-Ca^{2+} Release on Local and Global SR-Ca^{2+} Release in Ventricular Cardiomyocytes

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A brief statement explaining the importance of the results and why rapid publication is justified is given in the accompanying cover letter.

ABSTRACT

In heart muscle, the physiological function of IP_3-induced Ca^{2+} release (IP_3ICR) from the sarcoplasmic reticulum (SR) is still the subject of intense study. A role of IP_3ICR may reside in modulating Ca^{2+}-dependent cardiac arrhythmogenicity. Here we observe the propensity of spontaneous intracellular Ca^{2+} waves (SCaW) driven by Ca^{2+}-induced Ca^{2+} release (CICR) in ventricular myocytes as a correlate of arrhythmogenicity on the organ level. We observe a dual mode of action of IP_3ICR on SCaW generation in an IP_3R overexpression model. This model shows a mild cardiac phenotype and mimics pathophysiological conditions of increased IP_3R activity. In this model, IP_3ICR was able to increase or decrease the occurrence of SCaW depending on global Ca^{2+} activity. This IP_3ICR-based regulatory mechanism can operate in two "modes" depending on the intracellular CICR activity and efficiency (e.g. SCaW and/or local Ryanodine Receptor (RyR) Ca^{2+} release events, respectively): a) in a mode that augments the CICR mechanism at the cellular level, resulting in improved excitation-contraction coupling (ECC) and ultimately better contraction of the myocardium, and b) in a protective mode in which the CICR activity is curtailed to prevent the occurrence of Ca^{2+} waves at the cellular level and thus reduce the probability of arrhythmogenicity at the organ level.

INTRODUCTION

The presence of 1,4,5-trisphosphate-induced intracellular Ca^{2+} release (IP_3ICR) has been established in cardiomyocytes for some time [1] however its regulatory function in cardiac excitation-contraction coupling is still subject of ongoing debate.

In contrast to Ca^{2+}-induced Ca^{2+} release, IP_3ICR requires the intracellular synthesis of IP_3 triggered by G-protein-coupled receptor (GPCR) activation which is in turn induced by hormone binding. Although IP_3ICR may not strongly contribute to the global Ca^{2+} transient amplitude in ventricular myocytes, a more complex role of modulating CICR under conditions of cardiac pathophysiological remodeling has been suggested. Several cardiac pathologies, in particular Ca^{2+}-dependent pro-arrhythmogenicity (e.g. delayed after depolarizations) [1–3] have been associated with a functional up-regulation of IP_3 receptors (IP_3Rs). Moreover, evidence indicates that G protein-coupled receptor (GPCR) activation is time dependent and that IP_3ICR may have a different impact in the short versus the long term. In the short term, binding of Ca^{2+} to RyR could lead to increased open probability of the receptor (Ca^{2+}-dependent sensitization) in the presence of trigger Ca^{2+} provided by the opening of the L-type Ca^{2+} channel, thus facilitating SR-Ca^{2+} release; in the long term, depleted SR-Ca^{2+} stores could lead to an opposite, a Ca^{2+}-dependent desensitization of the RyR and thus to reduced RyR2 open probabilities. Consequently, in the latter respect, the CICR chain reaction would be impaired [4].

In this study we were able to provide evidence supporting this dual-action hypothesis by using an IP_3R overexpressing mouse model. The timing / duration of IP_3ICR interaction with the CICR machinery plays fundamentally a modulatory role for global SR-Ca^{2+} release events, e.g. Ca^{2+} wave appearance and/or Ca^{2+} wave speed. The dual regulatory mechanism presented in this study is a new facet of intracellular Ca^{2+} homeostasis that has not been described before, but which has a significant impact on the direction of the remodeling process and should be considered. In this manner the present study makes a contribution in the field for the understanding of global ECC remodeling in which IP_3-Ca^{2+} release plays a significant role.

MATERIAL AND METHODS

All experiments were approved by the State Veterinary Office of Bern, Switzerland, according to Swiss Federal Animal Protection Law and performed at room temperature (22°C). Ventricular myocytes were isolated from mice specifically overexpressing IP_3R type 2 in cardiac tissue [5]. More details about UV flash photolysis of caged IP_3 in saponin-permeabilized and intact ventricular myocytes are given in the supplementary material.

Each dot in a figure represents a measurement from an individual cell. The number of animals (N) and cells (n_c) are given in the figure legends. Statistical analysis was performed by

fitting a linear mixed-effects model with 1 level (isolation) to data and pairwise comparison with Benjamini–Hochberg correction was used to test significant differences among individual groups.

RESULTS AND DISCUSSION

We conducted experiments with permeabilized cardiomyocytes isolated from transgenic (TG) mice overexpressing (functional) IP_3R. This TG model was originally introduced by Nakayama et al. in 2010 [5] and is one of the few available models to study the role of IP_3ICR in Ca^{2+} signaling in cardiomyocytes in which the relative ratio of IP_3R and RyRs is significantly increased, mimicking some of the remodeling observed in several cardiac pathologies. It is characterized by a mild phenotype with an upregulation of functionally expressed IP_3R by about 13 times in ventricles [6] and was successfully used in recent studies [6,7].

The appearance of local SR Ca^{2+} release events and Ca^{2+} waves in response to acute global intracellular IP_3 concentration jumps were studied by UV-flash photolysis of caged IP_3. Uncaging of caged IP_3 will increase the global intracellular IP_3 concentration in the entire cell in a rapid ("acute") and homogeneous fashion within ~2 ms. To mimic a more "chronic" IP_3 response we alternatively used ET-1 to activate GPCR-induced intracellular IP_3 synthesis, which increases intracellular IP_3 levels orders of magnitude more slowly than UV photorelease. However, also protein kinase C (PKC) activation may be part of ET-1-GPCR pathway stimulation. Although PKC affects various intracellular targets, we demonstrated that ET-1-triggered arrhythmogenic responses could be suppressed by blocking IP3R2. This indicates that IP3ICR is the main mechanism for arrhythmic responses and that other PKC phosphorylation effects are minor in this setting.

In a series of control experiments, IP_3 photorelease triggered an increase in appearance of local Ca^{2+} release events ($p = 0.0010$) (Supplemental Figure 1). This acute response supports the idea of a boosting effect of IP_3ICR in triggering and shaping local Ca^{2+} events. A more detailed description is given in the supplements. The set of control experiments also indicates that the majority and surplus IP_3 triggered Ca^{2+} events have the spatio-temporal characteristics of Ca^{2+} sparks. In addition to the observable Ca^{2+} events (e.g. Ca^{2+} sparks, Ca^{2+} puffs) based on synchronized openings of clustered Ca^{2+} release channels, eventless SR-Ca^{2+} release may occur. This behavior is possible due to individual or non-functional clustered IP_3Rs openings [8].

Next, we focused on Ca^{2+} waves affected by IP_3ICR in ventricular myocytes. The underlying mechanisms of spontaneous Ca^{2+} waves are CICR and their occurrence under physiological conditions (1.8 mmol/l $[Ca^{2+}]_0$) is rather low. Pro-arrhythmogenicity on the organ level is connected with the occurrence of Ca^{2+} waves on the cellular level.

To investigate a potential modulatory role of IP_3ICR on Ca^{2+} waves the protocol shown in Figure 1A was applied to resting myocytes (Figure 1B,C). Since the SR-Ca^{2+} content represents a regulatory mechanism for CICR [9,10] intact ventricular cardiomyocytes were externally paced to keep the SR- Ca^{2+} content at comparable levels (Figure 1A) and loaded with an AM form of caged IP_3. Figure 1B shows examples of spontaneous Ca^{2+} waves before and after photorelease of IP_3. After IP_3 photorelease Ca^{2+} wave speed was promptly accelerated from 74.20 [68.39, 80.02] to 84.81 [74.99, 94.64] µm/s (mean [95% CI], p = 0.0150, Figure 1C) supporting the hypothesis of a boosting function of IP_3ICR for CICR in intact cardiomyocytes. In addition, no significant changes of Ca^{2+} wave amplitude, latency or frequency were detected (Supplemental Figure 2).

In Figure 2 an essentially identical experimental protocol was applied, now based on direct GPCR activation with the vasoconstrictor peptide endothelin-1 (ET-1) in order to generate IP_3 intracellularly in intact cardiomyocytes. This also mimics a more "chronic" IP_3 effect established in a time range of seconds to minutes.

In contrast to the acute IP_3 photorelease response, here the Ca^{2+} wave speed was unchanged. In addition to Ca^{2+} wave frequency, we also analyzed the latency to the appearance of the first Ca^{2+} waves after application of the SR-Ca^{2+} loading protocol. Unexpectedly, in the presence of ET-1, the cells appeared to respond differently, with some cells responding with a spontaneous Ca^{2+} wave frequency increase, while others appeared to show lower wave activity. Analysis of the control situation without ET-1 showed the following correlation with the ET-1 response: (1) responders with an initial low spontaneous Ca^{2+} wave activity showed a tendency to increase the frequency of Ca^{2+} waves in the presence of ET-1 stimulation and (2) responders with an initial high spontaneous Ca^{2+} wave activity showed the opposite behavior, a tendency to decrease the occurrence of Ca^{2+} waves and a longer latency period in the presence of ET-1 stimulation. We separated these two groups based on the change of spontaneous Ca^{2+} waves activity (SCaW) after application of ET-1 (Figure 2C,D), namely, on the observation that the mean SCaW activity of cells in the increasing group, is only half the mean SCaW activity of the decreasing group. ET-1 affects, in a dual manner, the SCaW frequency and latency depending on the initial SCaW activity. Low SCaW activity in control conditions leads to an increase of SCaW frequency (p = 0.0009) and a decrease of SCaW latency (p = 0.0545) in presence of ET-1. Opposite effects are induced when cells depart from a high frequency of SCaW. ET-1 decreases SCaW (p = 0.0002) and increases SCaW latency (p = 0.0024). These changes were prevented by 2-APB (Supplemental figure 3).

We have hypothesized that IP_3ICR in combination with additional regulatory factors may affect the Ca^{2+} dependent open probability of RyRs. In other words, shifting the Ca^{2+}-open probability curve of the RyRs to lower intracellular Ca^{2+} concentrations. IP3R2s and RyR2s are most likely co-expressed in cardiomyocytes and may form microclusters. However, RyR2s normally have a very low open probability (P_o) at resting cytosolic Ca^{2+} concentrations (i.e. at the

time of the diastole). During each action potential the P_o of the RyR2 is substantially increased by Ca^{2+} entering via L-type Ca^{2+} channels. Now, long lasting Ca^{2+} release by IP3R2s in the close micro-environment of RyR2s (or dyad) which is not synchronized by AP's, effectively increase the local [Ca^{2+}] above and beyond what it would be otherwise. This will result in a gain-of-function on the level of RyR2s. In other words, the RyRs P_o curve is left-shifted due to additional Ca^{2+} priming the local Ca^{2+} concentration. IP3Rs are not really more Ca^{2+} sensitive but they appear to be more sensitive. In addition, the timing and duration of IP_3ICR interference in regulating the Ca^{2+} sensitivity of RyRs openings appears to be of crucial importance for the modulatory effect on CICR and the global Ca^{2+} homeostasis in cardiomyocytes. In other words, the initial remodeling situation at the cellular level and time frame when IP_3ICR interacts/interferes with the CICR machinery is critical for the resulting modulatory effect of IP_3 on the global (cell-wide) Ca^{2+} signaling. On the one hand, the effect can be supportive for the overall CICR global transient formation in case of an insufficiently working CICR machinery, on the other hand it could work in a protective way (anti-arrhythmogenic) by increasing the SR-Ca^{2+} leak to keep the cardiomyocytes functioning as long as possible, even at the cost of reduced CICR efficiency. However, we have already shown that in WT animals the functional impact of IP3ICR on ECC is small, if not negligible. This situation changes under conditions of increased functional IP3R2 expression under pathophysiological conditions as seen in the used IP3R overexpression model used here. Taken together, our results suggest that increased expression of IP_3R in cardiomyocytes and the corresponding increased IP_3ICR in the context of ECC remodeling under pathophysiological conditions could have a dual action in the functional modulation of CICR. Based on their individual SCaW appearance and activity/history, respectively, cardiomyocytes can respond in essentially opposing ways: pro- or anti- arrhythmogenic with the consequence of boosting or curtailing CICR chain reactions. Depending on the stage and progression of the cardiac phenotype IP_3ICR could be a regulatory cellular mechanism switching between a supportive or protective "mode".

In conclusion, our findings support the hypothesis that IP_3-Ca^{2+} release can both support and inhibit the CICR mechanism. The latter mechanism helps protecting the myocardium from arrhythmogenic episodes or assists in triggering CICR to increase myocardial contraction. The direction of this regulation is largely determined by the initial Ca^{2+} level during the progression of cardiac disease and the corresponding myocyte remodeling.[7]

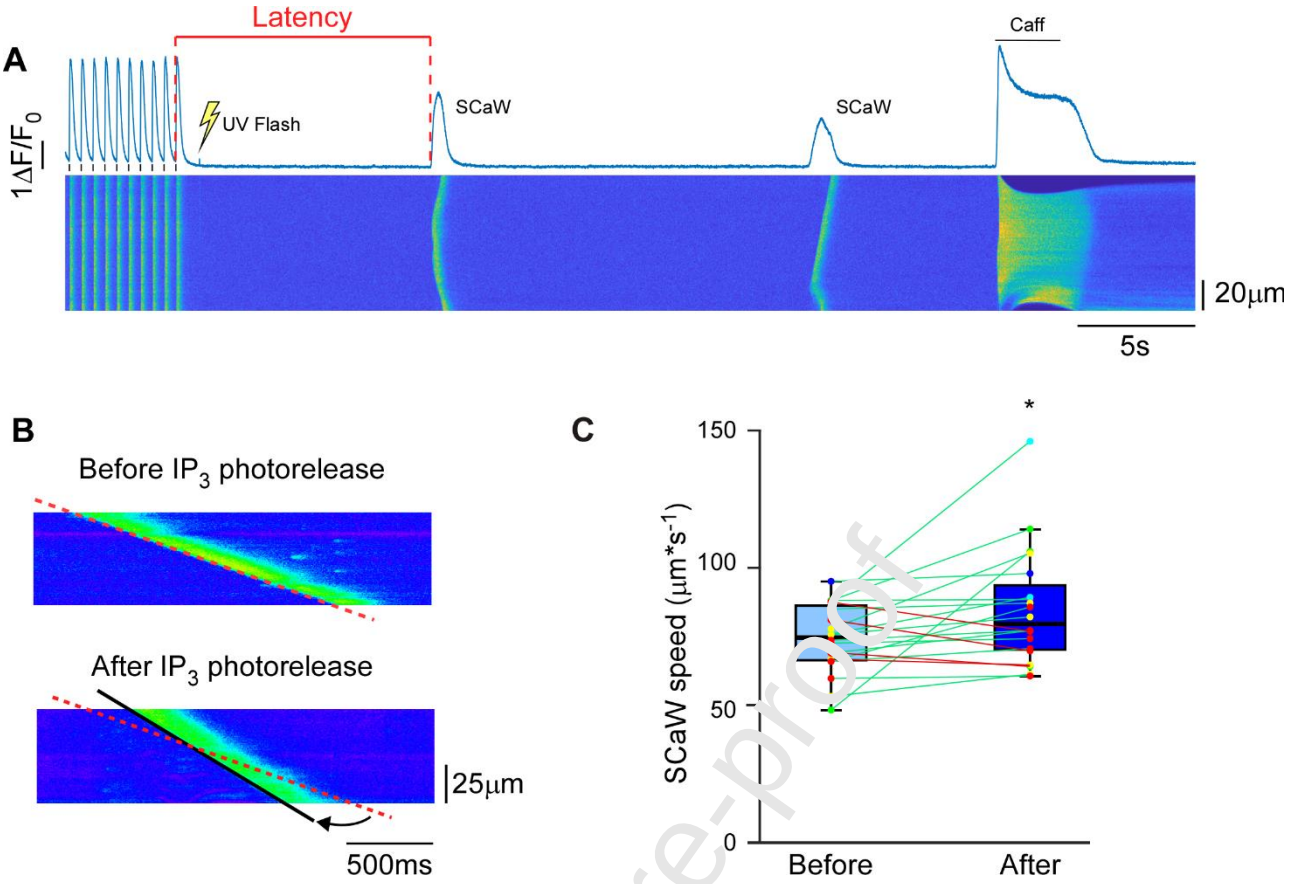

Figure 1. Spontaneous intracellular Ca^{2+} waves after UV-flash photolysis of caged IP_3 in TG intact ventricular myocytes. (A) Protocol applied to intact cardiomyocytes to examine SCaW parameters. After 30 s of 2 Hz external field stimulation SCaWs were detected for 30 s in control conditions or with UV flash applied 1s after last triggered Ca^{2+} transient. SR-Ca^{2+} load was assessed by 10 mmol/l caffeine. (B) Line scans showing SCaW before and after photolytic IP_3 release. (C) The Ca^{2+} wave speed significantly increases after IP_3 liberation by UV-flash. N = 6, N_c = 20, p-values of <0.05 are indicated by *.

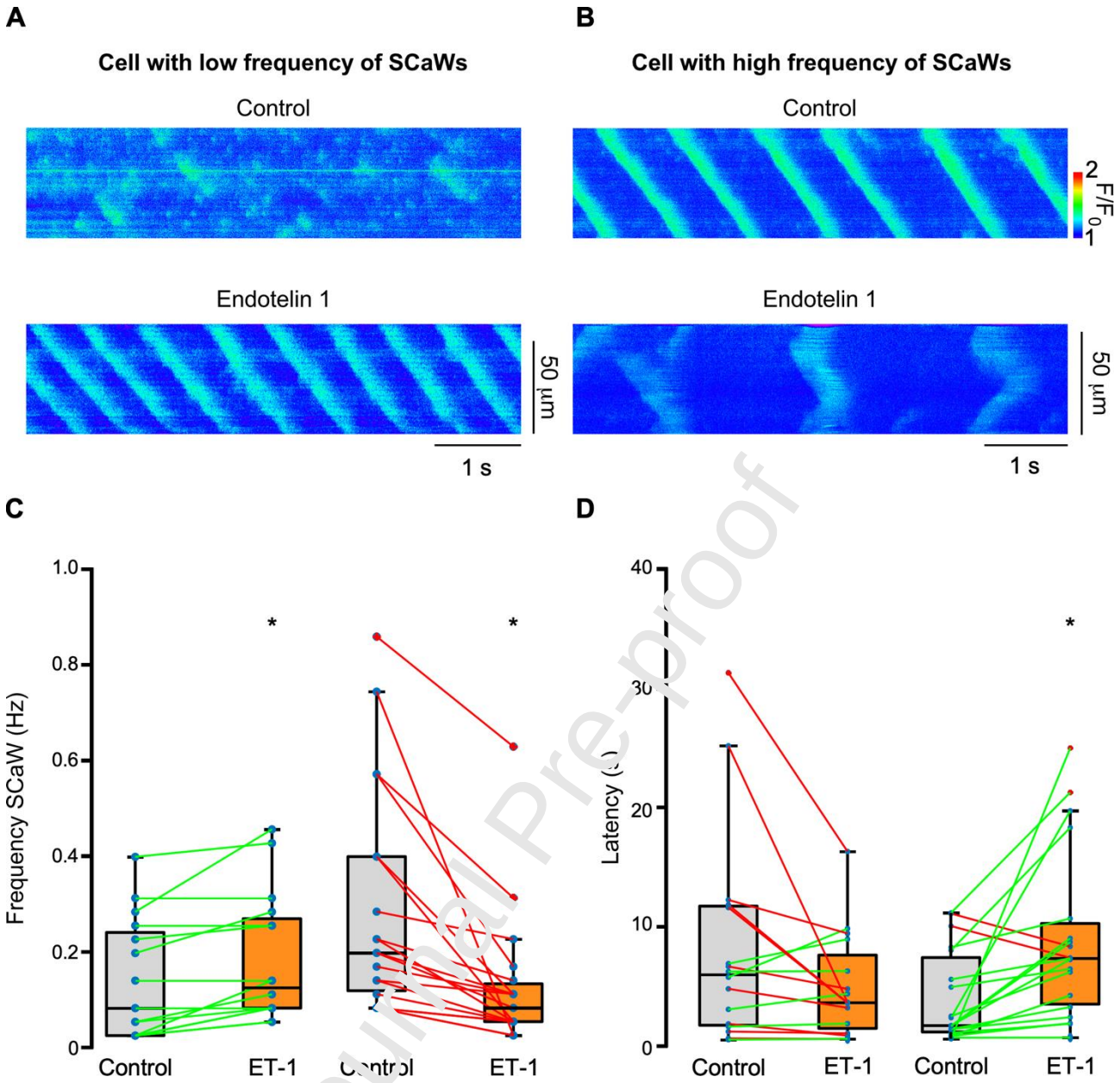

Figure 2. SCaW occurrence during ET-1 (100 nmol/l) stimulation depends on initial spontaneous Ca^{2+} wave activity. After identical SR-loading protocol cardiomyocytes show different SCaW activities. Subsequent ET-1 stimulation (5 minutes) lead to dual effect of Ca^{2+} wave activity. Cardiomyocytes were divided into two groups based on the change of SCaW activity after application of ET-1. In cell with a low SCaW frequency (A) the application of ET-1 induces an increase in the SCaW frequency (C) and decrease of latency (D). However, cardiomyocytes with a high SCaW frequency (B) in control conditions, experience a decrease of SCaW frequency after ET-1 application (C) and increase of latency (D). N = 9, N_c = 16, p-values of <0.05 vs. control are indicated by *.

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Disclosures / Conflict of Interest

None.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors did not use generative AI or AI-assisted technologies in the development of this manuscript.

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