From global to local: a new understanding of cardiac electromechanical coupling

Summary

Background. The coupling between depolarisation and contraction of cardiac myocytes is fundamental to the physiology and pathophysiology of the heart. This review describes how the coupling depends on the interaction between proteins in subcellular «microdomains».

Method. The review is based on the authors' own research and on a discretionary selection of articles found by means of a literature search in PubMed.

Results. Essential aspects of the physiology and pathophysiology of the heart must be understood in terms of the interaction between proteins in delimited parts of the cells. The significance of the binding protein ankyrin-B and the Ca^{2+} channel IP_3R (inositol 1,4,5 triphosphate receptor) is best understood in this context. Abnormal function of ankyrin-B and IP_3R is involved in congenital diseases with increased risk of arrhythmia and in weakened contractility and arrhythmias in connection with heart failure. The pathophysiological mechanism involves a change in Ca^{2+} homeostasis locally in the heart muscle cells.

Interpretation. Normal cardiac electromechanical coupling depends on control of ionic homeostasis in intracellular microdomains. Insight into the interaction between proteins in these «local neighbourhoods» provides new explanations for the pathophysiology of heart disease and paves the way for further research on arrhythmia mechanisms in hereditary diseases such as ankyrin-B syndrome.

Mathis Korseberg Stokke
m.k.stokke@medisin.uio.no
Frederic Rivelsrud
Ivar Sjaastad
Ole M. Sejersted
Fredrik Swift
Institute for Experimental Medical Research, Oslo University Hospital and University of Oslo, Oslo, Norway
and
KG Jebsen Cardiac Research Centre and Centre for Heart Failure Research, University of Oslo, Oslo, Norway

Since the publication of Sydney Ringer's seminal article in 1883, it has been known that Ca^{2+} plays a key role in the contraction of the heart (1). Research in the 1900s demonstrated that the contractility of the heart is adapted to the organism's needs through the regulation of the concentration of Ca^{2+} in the heart muscle cells. However, it took a hundred years before Ringer's discovery of this regulation was explained at molecular level. The electromechanical coupling of the heart has now been described in detail, but we still have no explanation for and cannot predict ordinary phenomena such as impaired contractility and arrhythmias in connection with heart failure.

This paper describes a cardiophysiological approach that has attracted growing attention over the past decade. The approach studies the interaction between proteins in delimited «microdomains» in cells. The binding protein ankyrin-B and the Ca^{2+} channel IP_3R (inositol 1,4,5 triphosphate receptor) are examples of proteins that probably have to be understood in this connection.

Method

The paper is based on the authors' own research in the field and on a discretionary selection of articles found by means of a literature search in PubMed. The following search terms were used: «*ANK2*», «ankyrin-B», «ankyrin-B syndrome», «Long QT syndrome type 4», «IP_3R». This resulted in 140 hits in PubMed on 15 February 2012.

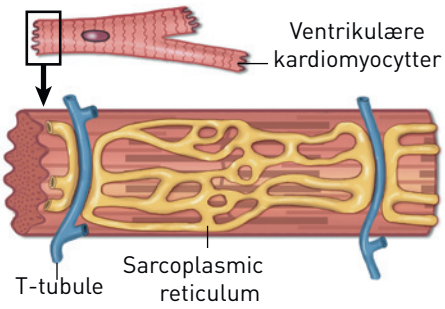
Since the focus of the article is on Ca^{2+} homeostasis in ventricular cells, we placed emphasis on articles that mainly concern ventricular cells or ventricular arrhythmias. The hypotheses formulated in the article concerning the function of ankyrin-B and IP_3R are our own, but in line with recent review articles in the field.

Classical understanding of the heart's electromechanical coupling

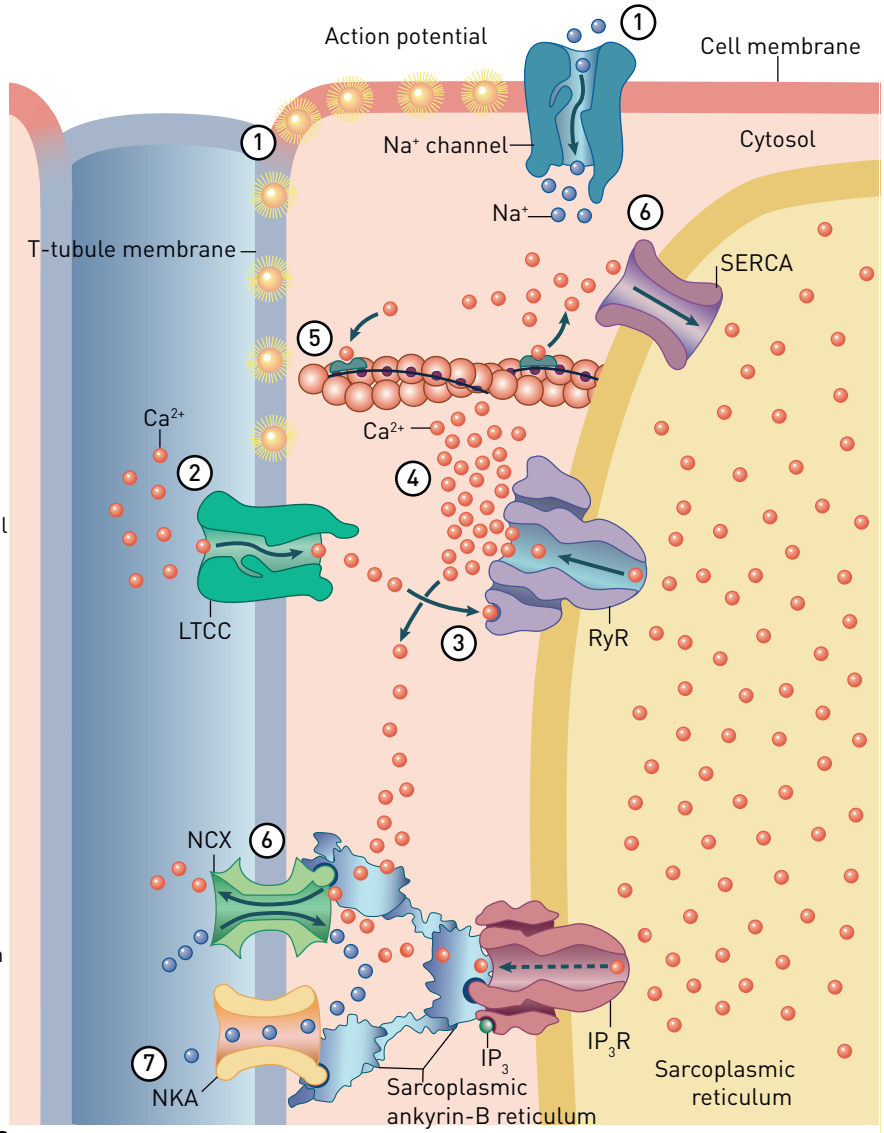
The theory of Ca^{2+} -induced Ca^{2+} release in the heart muscle cells describes a system for reinforcing the Ca^{2+} signal that has several control points (Fig. 1a) (2). On each activation (depolarisation) a small amount of Ca^{2+} is released into the heart muscle cells through L-type Ca^{2+} channels in the cell membrane. Entering Ca^{2+} binds to a Ca^{2+} release channel in the heart muscle cells' Ca^{2+} reservoir, the sarcoplasmic reticulum. These Ca^{2+} release channels, the ryanodine receptors (RyR), can be opened by a small amount of Ca^{2+} in the cytosol. When the RyR opens, Ca^{2+} flows out into the cytosol, where its concentration is therefore multiplied 5–15 times compared with the diastole. Ca^{2+} then binds to the contractile proteins and thereby starts the muscle contraction. The systolic concentration of Ca^{2+} in the cytosol determines the strength of the contraction. The coupling of Ca^{2+} and contraction thus have four important control points (3): First, the actual influx of Ca^{2+} through the cell membrane, which starts the process, is subject to regulation. This is important, since a large influx will result in larger releases from the Ca^{2+} reservoir. Second, the sensitivity of the RyR can be altered so that more Ca^{2+} is required for a large release. Third, the quantity of Ca^{2+} in the sarcoplasmic reticulum can be adjusted. This is an important control point, since the quantity of Ca^{2+} that is released on each activation increases exponentially with the quantity of Ca^{2+} in the sarcoplasmic reticulum. Fourth, the ability of the contractile proteins to react to Ca^{2+} can be adapted to the organism's need for an increased or reduced stroke volume.

Main points

- Precise, homogeneous and synchronous electromechanical coupling in individual cells forms the basis for normal heart function.
- The electromechanical coupling depends on the interaction between proteins in microdomains
- Ankyrin-B is a protein that safeguards the structure of such a microdomain.
- Reduced ankyrin-B function results in ankyrin-B syndrome or type 4 long QT syndrome



1. The action potential spreads rapidly in the cell membrane. The rapid depolarisation phase is due to the opening of Na^+ channels
2. The depolarisation opens L-type Ca^{2+} channels (LTCC) in the T-tubule membrane so that a small amount of Ca^{2+} is released into the cytosol
3. Ca^{2+} then binds to the Ca^{2+} released channels (RyR) in the sarcoplasmic reticulum (SR)
4. This releases a larger quatity of Ca^{2+}
5. Released Ca^{2+} binds to the contractile proteins
6. In the relaxation phase, some of the Ca^{2+} is pumped back into SR by a Ca^{2+} ATPase in SR (SERCA), while some is transported out of the cell by the Na^+/Ca^{2+} exchanger (NCX)
7. NCX locally regulates Na^+ and Ca^{2+} in interaction with Na^+ and K^+ AtPase in the cell membrane (NKA). The binding protein ankyrin-B brings about this interaction by binding to both NCX and NKA an thereby creating a micro-domain that also includes inositol 1,4,5 triphosphate receptor (IP_3R) in the SR-membrane



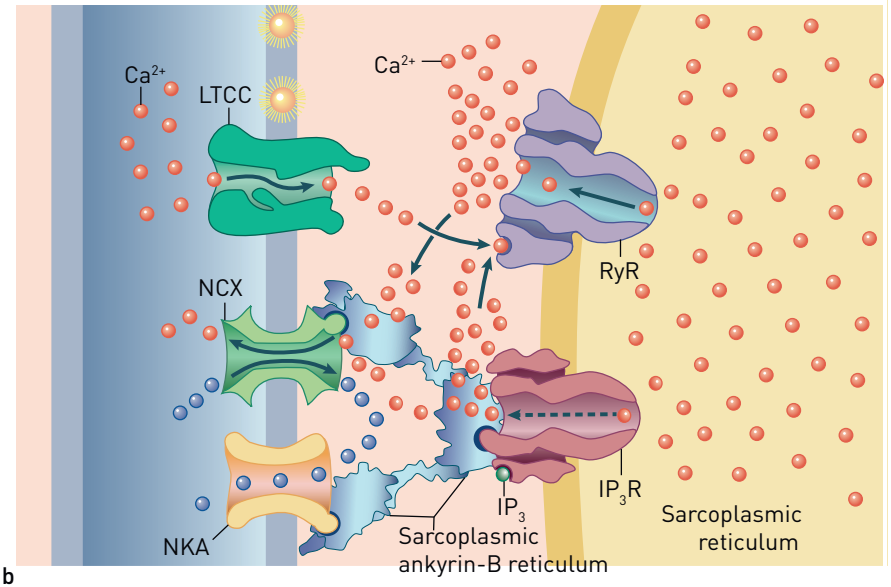


Figure 1 *a) Schematic diagram of the dyad and excitation-contraction coupling in ventricular cardiomyocytes. Solid arrows indicate ion fluxes in normal excitation-contraction coupling; broken lines mark IP_3-dependent Ca^{2+} flux. Note that NCX can transport both Ca^{2+} and Na^+ in both directions across the cell membrane, but that only Ca^{2+} flux out of the cell and Na^+ flux into the cell are marked on the figure. IP_3R can have two roles in the Ca^{2+} homeostasis of the cardiomyocytes. If IP_3R, NCX and NKA form an independent microdomain independent of RyR, as shown in a), this system may function as a safety valve where Ca^{2+} that is released through IP_3R is transported directly out through the Na^+/Ca^{2+}-ion exchange (NCX). This may prevent the Ca^{2+} concentration in SR from becoming too high. b) If IP_3R and the Ca^{2+} release channel RyR are co-localised, release from IP_3R may sensitise RyR to Ca^{2+} and potentiate Ca^{2+} release from the sarcoplasmic reticulum (SR), but also increase the probability of uncontrolled release.*

We have gradually learned much about these control points and how they are regulated in physiological and pathological situations. Over the past two decades, particular attention has been focused on RyR and its role in the pathophysiology of both weakened contraction and arrhythmias (4).

From «global» to «local» Ca^{2+} handling

The description of the connection between Ca^{2+} and contractions is based on an understanding of the interaction between proteins in the heart muscle cells. It has long been known that the diffusion of Ca^{2+} in cytosol is restricted because of the buffer effect of Ca^{2+}-binding proteins and geometrical obstacles (5). The diffusion coefficient of Ca^{2+} in water is 1 000 $\mu m^2/s$, whereas in cytosol it is estimated to be 10–20 $\mu m^2/s$ (6). Theoretically, such diffusion would still be enough for Ca^{2+} released from a single Ca^{2+} channel in the cell membrane to reach large parts of the cell in the course of one contraction cycle.

However, one problem with a system based on diffusion from few channels in the cell membrane would be large concentration differences within the cell and regional time differences for activation of the Ca^{2+} release channels. The extremely rapid (~1 ms from activation of the cell) and normally very homogeneous Ca^{2+} release in different areas points to a closer connection between membrane activation and Ca^{2+} release (7). There was therefore good reason to assume that depolarisation-contraction coupling must proceed very «locally», but at the same time in synchrony throughout the cell.

This process has largely been studied through phenomena that can be observed «globally», for example the total concentration of Ca^{2+} in the cytoplasm and the overall flux from all membrane channels. In the 1990s, however, improvements in microscope technology made it possible to describe the local processes in greater detail. Thus, it was confirmed that the rapid increase of Ca^{2+} in the cytosol when the heart muscle cells are activated is a result of many RyR channels opening simultaneously (8).

These RyR channels occur in well-defined «clusters» in the sarcoplasmic reticulum. L-type Ca^{2+} channels in the surface membrane are closely linked to a RyR cluster. This local structure constitutes the «dyad», the ultrastructural basic unit in the coupling between depolarisation and Ca^{2+} release in the heart muscle cells. The rapid spread of the action potential in the cell membrane (the whole cell is depolarised in the course of ~ 0.1 ms) (9) activates L-type Ca^{2+} channels in the whole cell almost simultaneously, and the close coupling with RyR in the dyads ensures synchronicity and homogenous Ca^{2+} release in the cell. It has been demonstrated that the dyad's structure can be disturbed, for example by heart failure, and that this results in heterogeneous, dyssynchronous and slower Ca^{2+} release (7, 10).

Dyads – one of many microdomains?

The structure of the dyad can thus explain essential physiological and pathophysiological phenomena associated with the Ca^{2+} homeostasis of heart muscle cells. However, the development of new molecular technologies has shown that the dyad is not the only local unit in the heart muscle cells, but rather one of a number of microdomains (11). An example of new discoveries that have accelerated this kind of thinking is the ankyrin-B syndrome, or long QT syndrome type 4.

A large number of sudden cardiac deaths as a result of arrhythmias in a French family led to genetic analyses with identification of mutations in the gene (*ANK2*) that encodes the ankyrin-B protein (12). Experimental animal studies later confirmed that a reduced quantity of ankyrin-B in the heart entails a higher risk for ventricular arrhythmias and sudden death triggered by increased sympathetic activity (13).

It was shown that cells from the ventricles of mice with a reduced amount of ankyrin-B more frequently depolarised spontaneously during the repolarisation phase (Phase3) and the resting phase (Phase 4) of the action potential. Such early and delayed afterdepolarisations may be caused by disturbances in Ca^{2+} homeostasis and spontaneous Ca^{2+} release from the sarcoplasmic reticulum that activates the Na^+/Ca^{2+} ion exchanger (NCX). The main task of NCX is to transport one Ca^{2+} ion out of the cell in exchange for three Na^+ ions during the cell's relaxation phase. This creates a net charge influx that has a depolarising effect on the cell membrane. This depolarisation is balanced by other ion fluxes having the opposite effect, and the surplus Na^+ is removed by the Na^+/K^+-ATPase. However, by releasing Ca^{2+} from the sarcoplasmic reticulum in phase 3 or phase 4 of the action potential, the NCX flux may depolarise the cell membrane sufficiently to trigger a spontaneous action potential. Spontaneous action potentials are seen as ventricular extrasystoles and may start arrhythmias. This causal chain of events was demonstrated during β-adrenergic stimulation in mice with reduced ankyrin-B: Spontaneous Ca^{2+} release caused afterdepolarisations, which triggered spontaneous action potentials. The mice also had an increased incidence of extrasystoles and sustained ventricular arrhythmias.

Not all mutations in *ANK2* predispose patients for arrhythmias; the clinical effect of the mutation depends on the significance of the mutation for the ankyrin-B *function* (14, 15). Despite this insight into the mechanism of the disease, prophylaxis of ankyrin-B syndrome is limited to β-adrenergic receptor agonists and implantable defibrillators, although other measures such as sympathetic denervation have also been proposed (16). More specific treatment will depend on the answer to the remaining question: How does reduced ankyrin-B function bring about changes in the Ca^{2+} homeostasis that increase the risk of arrhythmia?

Ankyrin-B and IP_3R – new kids on the block, or a whole new neighbourhood?

Ankyrin-B is a binding protein, ensuring that proteins involved in Ca^{2+} handling of heart muscle cells are retained in particular microdomains (17). Interestingly, proteins in the classic dyad are *not* among these. Thus, Ankyrin-B ensures local interaction between other proteins. The loss of this local interaction may be of great significance for Ca^{2+} homeostasis, at worst with fatal consequences. Thus more microdomains than the dyad have a bearing on Ca^{2+} handling.

Ankyrin-family proteins stabilise and bind proteins in the sarcolemma and the sarcoplasmic reticulum to the cytoskeleton of the cells (17). Ankyrin-B binds three proteins that are involved in Ca^{2+} homeostasis: NCX and the Na^+/K^+-pump (NKA) in the cell membrane and also the inositol 1,4,5 triphosphate receptor (IP_3R) in the sarcoplasmic reticulum (18) (Fig. 1a). It has long been known from the effect of digitalis that the interaction between NCX and NKA is essential for Ca^{2+} homeostasis in the heart: digitalis inhibits NKA, and therefore causes an accumulation of Na^+ in cytosol. This prevents NCX from exchanging Ca^{2+} with Na^+ from the extracellular fluid. As a result, the Ca^{2+} concentration in the cytosol increases. We have previously shown that NKA is co-localised with NCX (19, 20). As a result of the binding to ankyrin-B, these proteins form part of a microdomain with other proteins, including IP_3R. Interestingly, IP_3R is also a Ca^{2+} channel in the sarcoplasmic reticulum (21). Thus RyR is not the only route for Ca^{2+} release from the sarcoplasmic reticulum.

IP_3R is stimulated by IP_3, which is released when phospholipase C (PLC)-linked receptors in the cell membrane are activated by endothelin-1, angiotensin II or noradrenaline (22). The result is that IP_3R opens and releases Ca^{2+} from the sarcoplasmic reticulum to the cytosol. This process does not require prior depolarisation of the cell membrane. This Ca^{2+} release is therefore distinct from the Ca^{2+}-induced Ca^{2+} release from the RyR channels. In view of the fact that there are normally far fewer IP_3R than RyR, approx. 1: 50 (23), and Ca^{2+} release from each IP_3R is far slower and more limited than the Ca^{2+} release from RyR, one wonders what function the release from IP_3R has. This is an unanswered question, but there is reason to believe that the answer involves Ca^{2+} signalling in the IP_3R microdomain, and that the connection with heart disease may be due to internal changes in the latter or in the interaction with other microdomains (24).

IP_3R – regulator of RyR or safety valve?

Although it is known that IP_3R is also found in the membrane of the sarcoplasmic reticulum, its localisation in relation to the RyR

clusters remains undetermined. The localisation and functional interaction with RyR are crucial for the significance of abnormal IP_3R function in the ankyrin-B syndrome and heart failure.

We have formulated two hypotheses regarding the role of IP_3R (Fig. 1a, Fig. 1b): If IP_3R is co-localised with RyR, it is possible that it plays a part as regulator of the Ca^{2+}-induced Ca^{2+} release. Ca^{2+} release from IP_3R can change the local Ca^{2+} concentration around RyR and thereby influence RyR's sensitivity to Ca^{2+} (Fig. 1b). This would be an example illustrating that an interaction between different proteins in the same microdomain in the heart muscle cells can result in more precise regulation of the contraction.

Alternatively, IP_3R may play a part as a «safety valve» in the sarcoplasmic reticulum (Fig. 1a): Localisation of IP_3R in the same microdomain as NKA and NCX means that Ca^{2+} released through IP_3R can be easily transported out of the cell in a controlled manner. It is well known that accumulation of Ca^{2+} in the heart muscle cells has a positive effect in the form of increased inotropy. But if the accumulation becomes too large, Ca^{2+} will «leak» in an uncontrolled manner from the sarcoplasmic reticulum through RyR. As previously described, such RyR leakage could activate NCX, lead to afterdepolarisations and trigger arrhythmias. In recent years, however, it has been found that leakage from the sarcoplasmic reticulum may also take place «silently», independently of RyR (25). It is conceivable that such leakage could take place through IP_3R. It is therefore very interesting that heart failure, the end-point of many heart diseases, is associated with an increased amount of IP_3R in the heart (22, 26).

Heart failure is also associated with increased activity in the sympathetic nervous system and in the endothelin and renin-angiotensin systems. Since this means increased production of IP_3, IP_3R and its microdomain may play a larger part in heart failure than in healthy hearts (22). At present it is not known whether this is a protective mechanism to prevent over-filling of the sarcoplasmic reticulum, whether it contributes to weakened contractility by reducing the Ca^{2+} reservoir, or whether it increases the risk of arrhythmias by making RyR «hypersensitive». Clearly, both this and other microdomains in heart muscle cells should be studied further to enable an understanding of how the heart functions under normal circumstances and during illness.

Conclusion

Since Ringer's discovery of the importance of Ca^{2+} for muscle contraction, heart physiology has explained an increasing number of physiological phenomena by moving the focus from organ to cell to protein level. The research of recent decades has demonstrated the need to also study structure and function in delimited and adjacent microdomains in heart muscle cells. Examples of new insight into the significance of ankyrin-B and IP_3R show that these may be of great importance to an understanding of diseases with weakened contractility and increased risk of arrhythmias.

Mathis Korseberg Stokke (born 1977)
Doctor in specialist training and post-doctoral fellow at the Institute for Experimental Medical Research, Oslo University Hospital. He conducts research on basic mechanisms for arrhythmias, and is the co-author, with Ole Sejersted, of a textbook chapter on the electrophysiology of the heart. He is also employed in an administrative position at the Centre for Heart Failure Research.
The author has completed the ICMJE form and reports no conflicts of interest.

Frederic Rivelsrud (born 1982)
Junior Registrar in the Department of Surgery, Vestfold Hospital, Tønsberg. Former research assistant at the Institute for Experimental Medical Research at Oslo University Hospital.
The author has completed the ICMJE form and reports no conflicts of interest.

Ivar Sjaastad (born 1960)
Cardiologist with special expertise in experimental cardiology. Professor and Senior Consultant at the Institute for Experimental Medical Research.
The author has completed the ICMJE form and reports no conflicts of interest.

Ole M. Sejersted (born 1947)
Professor of Experimental Medicine. Head of Department at the Institute for Experimental Medical Research and second-in-command of the Division of Cardiovascular and Pulmonary Disease at Oslo University Hospital.
The author has completed the ICMJE form and reports no conflicts of interest.

Fredrik Swift (born 1977)
Cand. scient, PhD and post-doctoral fellow at the Institute for Experimental Medical Research, Oslo University Hospital/University of Oslo. Also employed in an administrative position at the Centre for Heart Failure Research.
The author has completed the ICMJE form and reports no conflicts of interest.

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