REGULATION OF CARDIAC EXCITATION-CONTRACTION COUPLING: A CELLULAR UPDATE

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The primary purpose of this paper is to present a basic overview of some "relatively" new ideas related to the regulation of cardiac performance and underlying excitation-contraction (EC) coupling that have yet to be incorporated to textbooks currently used for introductory graduate-level physiology courses. Within the context of cardiac EC coupling, this review incorporates information on microdomains and local control theory, with particular emphasis on the role of Ca2+ sparks as a key regulatory component of ventricular myocyte contraction dynamics. Recent information pertaining to Ca2+ release mechanisms specific to the sarcoplasmic reticulum is also presented, as well as the idea of the ryanodine receptor as a macromolecular signaling complex. Because of the potential relationship to maladaptive functional responses under conditions of cardiovascular pathology, the regulatory role of cardiac adrenergic and additional G protein-coupled receptors known to regulate cardiac function is included, and fundamental concepts related to intracellular signaling are discussed. Finally, information on the roles of vascular and cardiac nitric oxide as an important regulator of cardiac performance is included to allow students to begin to think about the ubiquitous role of nitric oxide in the regulation of the cardiovascular system. An important point of emphasis is that whole organ cardiac dynamics can be traced back to the cellular events regulating intracellular Ca2+ homeostasis and as such provides an important conceptual framework from which the students can begin to think about whole organ physiology in health and disease.

Key words: review; myocardium; Ca2+ sparks; G protein-coupled receptors; ryanodine receptor; nitric oxide

INCORPORATING NEW INFORMATION: WHAT DO WE TEACH?

With the advent of transgenic animals and novel experimental approaches, there has been an explosion of ideas and information in recent years related to the cellular regulation of cardiac contractile performance. However, much of this information remains controversial and unproven in human models and as such poses a problem when the question is asked: “What do we teach our graduate students in an introductory course on cardiovascular physiology?” Often, we are limited to 8–12 lectures to cover the entire cardiovascular system within a team-taught format, and no single textbook is adequate (nor could one possibly be) to address state-of-the-art conceptual information.
Thus the dilemma we are faced with as instructors is providing students with fundamental concepts and classical information while still providing exposure to new ideas. Toward this end, I have adopted a combined approach of utilizing the recently revised cardiovascular learning objectives associated with the Medical Physiology Curriculum Objectives Project adopted by the American Physiological Society (APS; http://www.the-aps.org/education/MedPhysObj/) in conjunction with supplementing assigned textbook readings with recent comprehensive review articles on cardiac excitation-contraction (EC) coupling. This approach has allowed for prioritization of necessary core concepts while ensuring necessary exposure to evolving information to expand currently accepted dogma on traditional cardiovascular theory.

The information in the present review is limited to concepts related to the regulation of cardiac performance not yet incorporated into commonly used cardiovascular textbooks as well as strategies to incorporate this new information within the framework of teaching traditional pump function and myocyte cellular dynamics. The slide presentation associated with this material can be found on the APS Education website (http://www.the-aps.org/education/refresher/MuscleRefresherCourse.htm), and the reader is referred to papers from past APS Refresher Courses on the teaching of traditional pump regulation and related physiology previously published in this journal (14).

WHOLE BODY OXYGEN CONSUMPTION AS INTEGRATOR

A prevailing theme throughout my own lectures is that whole organ cardiac physiology can be traced back to the cellular events underlying a single heart beat. Specifically, I emphasize the point that mechanisms that alter cytosolic Ca$^{2+}$ and/or myofilament Ca$^{2+}$ sensitivity will alter left-ventricular (LV) developed force. Thus control of cardiac function ultimately occurs at the cellular level, and in this case the whole is truly the sum of the parts. Within the context of utilizing an integrative approach to teaching cardiovascular physiology and the regulation of cardiac performance, one approach is to emphasize a "forest" view of the heart by first presenting the Fick equation for whole body oxygen consumption, as follows

$$V_{O_2} = \text{cardiac output} \times a \cdot V_{O_2\text{diff}}$$

where cardiac output = heart rate $\times$ stroke volume and $a$ is arterial.

At its simplest level, one can then talk about the physiological (systems) response to stresses, such as exercise, and subsequent alterations in cardiac output in terms of regulation of the heart rate and both the intrinsic and extrinsic mechanisms by which the stroke volume is regulated. At this point, it is important to stress the fact that true changes in cardiac contractility are independent of changes in preload and afterload, which also helps to emphasize the importance of adrenergic regulation on cardiac performance. With this scheme provided at the outset, it becomes easier to transition back to the role of transsarcolemmal Ca$^{2+}$ fluxes as a fundamental component to underlying cardiac receptor signaling, pharmacology, and functional responses in health and disease.

MICRODOMAINS AND LOCAL CONTROL OF EC COUPLING

Central dogma of EC coupling. For the purposes of brief review, Fig. 1A depicts the cellular events that underlie a single heart beat and illustrate phenomena related to global transsarcolemmal Ca$^{2+}$ fluxes within a single ventricular myocyte. The central dogma underlying cardiac EC coupling includes the theory of Ca$^{2+}$-induced Ca$^{2+}$ release, whereby small increases in Ca$^{2+}$ in the vicinity of the sarcoplasmic reticulum (SR) lead to much larger Ca$^{2+}$ release from internal stores (SR). Upon membrane depolarization, Ca$^{2+}$ enters the cytosol primarily through a voltage-gated L-type Ca$^{2+}$ current ($I_{Ca}$), which triggers Ca$^{2+}$ release from the SR via Ca$^{2+}$ release channels (ryanodine receptor 2, RyR2). Calcium is then free to bind to troponin C and engage the contractile machinery. We also know that small amounts of Ca$^{2+}$ can enter the cell through the Na/Ca exchanger.

The cartoon presented in Fig. 1A is also useful to illustrate the close proximity of the voltage-gated Ca$^{2+}$ channels (VGCCs) to the RyR. The t-tubules are found nearly every 1.5 μm along the z-line and penetrate the heart cell like spokes on a wheel; along the intracellular side of the t-tubules are the SR terminals with clusters of RyRs spanning the 10- to 20-nm gap between the t-
FIG. 1. Cardiac excitation-contraction (EC) coupling events. A: electrical excitation at the sarcolemmal membrane activates voltage-gated Ca\(^{2+}\) channels (\(I_{Ca}\)), and the resulting Ca\(^{2+}\) entry activates Ca\(^{2+}\) release from the sarcoplasmic reticulum (SR) via ryanodine receptors (RyRs), resulting in contractile element activation. At this point, a useful instructional paradigm is to ask students to imagine a diagonal line through the myocyte, whereby the processes below the line represent Ca\(^{2+}\) influx mechanisms and above the line are processes related to Ca\(^{2+}\) efflux. For relaxation to occur, cytosolic Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) must decline, allowing Ca\(^{2+}\) to dissociate from troponin. This involves transport of Ca\(^{2+}\) out the cytosol by 1 of 4 pathways: the SR Ca\(^{2+}\)-ATPase (SERCA) pump located on the SR, a sarcolemmal Na/Ca exchanger (NCX), a sarcolemmal Ca\(^{2+}\)-ATPase, and a mitochondrial uniporter. The SERCA pump is quantitatively the most important cellular mechanism for Ca\(^{2+}\) removal, hence, relaxation, and, depending on the species of interest, can account for \(\sim 70\%\) of Ca\(^{2+}\) removal, whereas the Na/Ca exchanger accounts for \(\sim 28\%\) of Ca\(^{2+}\) removal. PLB, phospholamban; Mito, mitochondria. B: this slide can also be used to illustrate the point that Ca\(^{2+}\) is required to activate the myofilaments and does so in a cooperative manner [modified from Bers (2) with permission].
mechanisms that alter cytosolic Ca$^{2+}$/H1001, which would raise the cytosolic Ca$^{2+}$ concentration (i.e., [Ca$^{2+}$]) to ~600 nM. Again, I stress the point that mechanisms that alter cytosolic Ca$^{2+}$ and/or myofilament sensitivity alter developed force, which helps lay the foundation for adrenergic regulation of cardiac contraction because it is known that the VGCC, RYR, phospholamban, and troponin I (TnI) can all be phosphorylated by the cAMP-dependent protein kinase (PKA) subsequent to β-adrenergic activation (see below and Fig. 4).

**Ca$^{2+}$ sparks underlie the Ca$^{2+}$ transient ([Ca$^{2+}$])**. Rarely mentioned in current general physiology textbooks is the idea of Ca$^{2+}$ sparks as an underlying basis for the [Ca$^{2+}$]. In their landmark paper, Cheng et al. (5) identified Ca$^{2+}$ sparks as the elementary events underlying Ca$^{2+}$ release in ventricular myocytes, thereby providing support for the local-control theory of cardiac EC coupling. Thus an important point of emphasis to students is that, whereas the [Ca$^{2+}$], that controls contraction is largely a global process (cellwide), as illustrated in Fig. 1, Ca$^{2+}$ release from the SR is controlled largely at the local (subcellular) level. Importantly, normal EC coupling involves a well-ordered and stereotyped sequence of events that includes Ca$^{2+}$ sparks, and Ca$^{2+}$ sparks are currently thought to be the fundamental units of SR Ca$^{2+}$ release occurring both at rest and during contractile activation.

A representative example of Ca$^{2+}$ sparks and their measurement is illustrated in Fig. 2, A–D, and it is clear from this example that Ca$^{2+}$ sparks do indeed occur at the level of the t-tubule (Fig. 2E). One useful illustration in teaching local-control theory is that Ca$^{2+}$ sparks represent Ca$^{2+}$ passing through RyRs and are small, local Ca$^{2+}$ release events that can be evoked by depolarization and action potentials, respectively. It is important to emphasize that, although Ca$^{2+}$ sparks remain incompletely understood, the rationale for inclusion in basic cardiovascular lectures is as follows: 1) summation of Ca$^{2+}$ sparks provides the microscopic basis of the [Ca$^{2+}$]; 2) Ca$^{2+}$ sparks provide an explanation for graded contractions, which are modulated by local control; 3) Ca$^{2+}$ sparks provide insight into microdomains and where they occur; and 4) Ca$^{2+}$ sparks provide insight into pathological changes in EC coupling, such as Ca$^{2+}$ waves and cardiac arrhythmias.

**RyR2 as a calcium release channel and macromolecular signaling complex.** Other emerging concepts worthy of incorporation into lectures on cardiac EC coupling include some degree of focus on RyR2 as a phosphorylation target and a macromolecular signaling complex. Specifically, although it is widely accepted that RyR2 serves as a Ca$^{2+}$ release channel in cardiac muscle, it is less appreciated that RyR2 can be phosphorylated by a variety of kinases, including PKA, protein kinase C, protein kinase G, and calcium-calmodulin-dependent protein kinase II. The details of this regulation continue to evolve; however, presentation of this information illustrates the point that RyR2 is yet another important site of regulation within the cardiac myocyte.

With regard to the concept of RyR2 as a macromolecular signaling complex, RyR2 can function as a scaffolding protein, localizing numerous key regulatory proteins including kinases, phosphatases, and adaptor/anchoring proteins to the junctional complex (Fig. 3). Specific proteins thought to be involved in this macromolecular signaling complex include the FK506-binding protein FKBP12.6 (a member of the immunophilin family and known receptor for immunosuppressant drugs including rapamycin and FK506), the catalytic and regulatory subunits of PKA, muscle A kinase anchor proteins, and the protein phosphatases PP1 (anchored by spinophilin) and PP2A (anchored by PR130). That control of RyR2 function is under the influence of local signaling complexes has led to the idea of the macromolecular signaling complex allowing for a graded physiological response to stress.

So what is the role of FKBP12.6 on RyR2 function? FKBP12.6 is bound to and regulates the RyR (Fig. 3), and it is thought to stabilize the RyR in a closed confirmation, thereby preventing diastolic Ca$^{2+}$ leak. Upon phosphorylation by PKA, FKBP12.6 dissociates from the RyR2, resulting in increased channel activity (i.e., open probability, $P_o$). FKBP12.6 has also been implicated in the phenomenon of “coupled gating” between two neighboring RyR2 channels, and there is...
some thought that FKBP12.6 may also play a role in regulating \(Ca^{2+}\) sensitivity of RyR2 (i.e., when FKBP12.6 is low, RyR2 is more sensitive to activation by \(Ca^{2+}\)). Of particular clinical interest is the potential physiological relevance of maladaptive responses in this regulatory complex under specific pathological conditions. Specifically in the failing heart, hyperphosphorylation of RyR2 by PKA subsequent to increases in circulating catecholamines results in the dissociation of FK506 from RyR2, the consequences of which include depletion of SR \(Ca^{2+}\) and aberrant release of \(Ca^{2+}\) during diastole, which can trigger fatal
RyR as a macromolecular signaling complex. RyR is a tetrameric structure comprising 4 subunits of ~600,000 Daltons each, which yields a molecular mass for a single channel of ~2.3 million Daltons. RyR2 is specific to cardiac muscle. Proteins thought to be involved in the macromolecular signaling complex include FKBP12.6, the catalytic and regulatory subunits of PKA, muscle anchoring proteins for A kinase (AKAP), protein phosphatase 1 (PP1), and PP2A (PR130). UNK, unidentified target proteins; RII, type II receptor [reprinted from Marks (9) with permission].

cardiac arrhythmias. Aberrations in this macromolecular complex in the failing heart may also provide insight into the clinical utility of β-adrenergic blockade on improved survival in patients with congestive heart failure, i.e., restoration of RyR2 channel function toward normal by reversing the hyperphosphorylated state of RyR2.

EXTRINSIC REGULATION OF THE INOTROPIC STATE: β-ADRENERGIC SIGNALING AND BEYOND

The schema presented in Fig. 4 provides a unifying framework for the idea of intrinsic and extrinsic mechanisms by which the contractile state of the myocardium can be modified. Because the Frank-Starling and force-frequency effects are fairly well characterized in most textbooks, the primary focus of the final two sections of this paper addresses mechanisms of adrenergic regulation of the inotropic state and highlights the role of additional mediators, including those released by the endothelium that modulate muscle function. Well-known effects of sympathetic nervous system (SNS) stimulation on cardiac β1-adrenergic receptors (ARs) include both enhanced contraction and relaxation mediated by PKA phosphorylation of the VGCC, RyR, TnI, and phospholamban, respectively, and subsequent effects on intracellular Ca2+ homeostasis (Fig. 5). However, the contribution of additional adrenergic and other G protein-coupled receptors (GPCRs) on intracellular signal transduction and associated cardiac contraction may assume in-

FIG. 3.
RyR as a macromolecular signaling complex. RyR is a tetrameric structure comprising 4 subunits of ~600,000 Daltons each, which yields a molecular mass for a single channel of ~2.3 million Daltons. RyR2 is specific to cardiac muscle. Proteins thought to be involved in the macromolecular signaling complex include FKBP12.6, the catalytic and regulatory subunits of PKA, muscle anchoring proteins for A kinase (AKAP), protein phosphatase 1 (PP1), and PP2A (PR130). UNK, unidentified target proteins; RII, type II receptor [reprinted from Marks (9) with permission].

FIG. 4.
EC coupling phosphorylation targets of β-adrenergic receptor (AR) stimulation. M3-Rec, muscarinic receptor; AC, adenylyl cyclase [reprinted from Bers (2) with permission].
increasing importance under a variety of clinical circumstances.

**Signal transduction and myocardial performance.** Often, students are under the impression that β₁-ARs are the only receptors involved in mediating SNS effects on cardiac contractility. Although the β₁-AR is clearly the predominant GPCR in the heart, it is not the only receptor regulating inotropic effects. Table 1 provides a simplified signal transduction scheme of GPCRs known to be present in the heart and illustrates cellular compartmentalization of agonist-induced effects on cardiac contraction. β₁, β₂, and α₁-ARs are all present in the myocardium and exert varying degrees of cardiac inotropy. The β₁-AR is coupled to G_s and results in the activation of PKA. The β₂-AR results in dual coupling to both stimulatory and inhibitory G proteins, G_s and G_i, and represents an elegant example of how redundancy in cellular signaling can simultaneously initiate and terminate an activator signal and allow for fine-tuning of LV contractile performance. The α₁-AR receptor is coupled to G_q, as are the GPCRs for angiotensin II and endothelin, which results in the production of the second messenger, diacylglycerol (DAG), and inositol trisphosphate (IP₃).

**TABLE 1**

<table>
<thead>
<tr>
<th>Receptor</th>
<th>β₁</th>
<th>β₂</th>
<th>α₁A/B/D/ANG II/ET</th>
<th>M₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary G protein</td>
<td>Gₛ</td>
<td>Gₛ/G₁</td>
<td>Gₛ/G₁1</td>
<td>G₁</td>
</tr>
<tr>
<td>Tissue distribution</td>
<td>Heart</td>
<td>Heart, lung, vessels, kidney</td>
<td>Heart, vessels, smooth muscle</td>
<td>Heart</td>
</tr>
<tr>
<td>Primary effector in heart tissue</td>
<td>AC</td>
<td>AC</td>
<td>PLC-β</td>
<td>AC</td>
</tr>
<tr>
<td>Endogenous agonist</td>
<td>NE, Epi</td>
<td>NE, Epi</td>
<td>NE, Epi, ANG II, ET</td>
<td>ACh</td>
</tr>
</tbody>
</table>

M₂, muscarinic; AC, adenyl cyclase; PLC, phospholipase C; P3K, phosphoinositol-dependent kinase 4; ET, endothelin; Epi, epinephrine; NE, norepinephrine; ANG II, angiotensin II; MAPK, mitogen-activated protein kinase; DAG, diacylglycerol; IP₃, inositol trisphosphate [modified from Rockman et al. (12) with permission].
messengers inositol triphosphate and diacylglycerol and activation of PKC with subsequent effects on both cardiac inotropy and cardiac growth. At this point, our students have been exposed to basic concepts of cell and membrane physiology, including basic intracellular signaling and receptor function, which provides an easy transition to cardiac receptors important to the regulation of the inotropic state. Furthermore, since the students are equally familiar with cardiac EC coupling, effects of receptor stimulation on EC-coupling dynamics and Ca\textsuperscript{2+} homeostasis become evident.

Desensitization and protein scaffolds. Although we usually place emphasis on receptor activation in relation to regulation of the inotropic state, little time is spent addressing how activation signals are terminated. In this regard, classical mechanisms of heterologous (nonagonist) receptor desensitization include receptor downregulation and/or phosphorylation. However, homologous (agonist-specific) receptor desensitization also occurs as a result of uncoupling of the β-AR from its G protein by activation of G protein-coupled receptor kinase 2 (GRK2, aka β-adrenergic receptor kinase), whereby GRK2 phosphorylates agonist-occupied receptors (Fig. 6). Once activated, β-arrestin is recruited to the membrane and sterically inhibits further G protein receptor coupling, and this mechanism has been implicated in the well-characterized desensitization of β\textsubscript{1}-ARs in the failing human heart. Interestingly, β-arrestin can also act as a protein scaffold to recruit clathrin to allow for endocytosis of activated receptors as well as interact with Src and promote activation of extracellular-regulated kinase signal transduction pathways. This dual signaling sheds light on possible mechanisms of receptor cross talk and, in the case of the heart, provides insight into how one receptor, such as the α\textsubscript{1}-AR, can induce both inotropic and growth effects, respectively.

NITRIC OXIDE AS MEDIATOR OF CARDIAC PERFORMANCE

In the past 10 years, emerging evidence has identified nitric oxide (NO) as an important determinant of the cardiac inotropic state (for recent review see Refs. 3, 4, and 11). Although incompletely understood, NO has been implicated in both positive and negative inotropic effects, and there is growing consensus that the effects of NO are indeed biphasic, concentration dependent, and stimulation specific. Importantly, recent experimental evidence suggests that the machinery for endogenous NO production may reside within the cardiac myocyte (as opposed to release from surrounding cardiac or vascular endothelium) and may subserve important SR Ca\textsuperscript{2+} release functions in response to stretch. One logical implication of this observation is a partial explanation for the well-characterized Frank-Starling effect in cardiac muscle. Equally intriguing is the speculation that neuronal NO synthase (nNOS) may subserve a Ca\textsuperscript{2+} reuptake function and exert important regulatory effects on sarco(endo)plasmic reticulum Ca\textsuperscript{2+}-ATPase. Important cross talk between NO and the β\textsubscript{1}-AR-signaling pathway is also known to impact the inotropic state, whereby NO is likely to exert important inhibitory ef-

![Mechanisms of homologous desensitization and cellular compartmentalization by G protein-coupled receptor kinase 1 (GRK2), JNK3, c-jun NH\textsubscript{2}-terminal kinase 3; MKK, mitogen-activated protein (MAP) kinase kinase; ASK1, apoptosis signal-regulating kinase 1; ERK, extracellular signal-regulated kinase [reprinted from Rockman et al. (12) with permission].](image-url)
fects on β₁-AR-mediated contraction. In this regard, endothelial NOS (eNOS) knockout mice demonstrate potentiated contractile responses following catecholamine exposure compared with nontransgenic control animals. Finally, transgenic manipulation of eNOS levels has also led to the discovery of NO as an important determinant of cardiac mitochondrial respiration, and both eNOS and inducible NOS (iNOS) have been implicated in cardioprotective effects under conditions of ischemic-reperfusion injury and the late phase of ischemic preconditioning. The complex and potentially important role of NOS isoforms on the regulation of cardiac performance in both health and disease awaits clinical validation; however, the significance of this regulation on cardiac function should not be underestimated.

DISCLOSURES

This work was supported by National Institute on Aging Grant K01 AG-00875.

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Submitted 11 August 2003; accepted in final form 19 August 2003

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