From syncitium to regulated pump: a cardiac muscle cellular update

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Korzick DH. From syncitium to regulated pump: a cardiac muscle cellular update. Adv Physiol Educ 35: 22–27, 2011; doi:10.1152/advan.00119.2010.—The primary purpose of this article is to present a basic overview of some key teaching concepts that should be considered for inclusion in an six- to eight-lecture introductory block on the regulation of cardiac performance for graduate students. Within the context of cardiac excitation-contraction coupling, this review incorporates information on Ca\textsuperscript{2+} microdomains and local control theory, with particular emphasis on the role of Ca\textsuperscript{2+} sparks as a key regulatory component of ventricular myocyte contraction dynamics. Recent information pertaining to local Ca\textsuperscript{2+} cycling in sinoatrial nodal cells (SANCs) as a mechanism underlying cardiac automaticity is also presented as part of the recently described coupled-clock pacemaker system. The details of this regulation are emerging; however, the notion that the sequestration and release of Ca\textsuperscript{2+} from internal stores in SANCs (similar to that observed in ventricular myocytes) regulates the rhythmic excitation of the heart (i.e., membrane ion channels) is an important advancement in this area. The regulatory role of cardiac adrenergic receptors on cardiac rate and function is also included, and fundamental concepts related to intracellular signaling are discussed. An important point of emphasis is that whole organ cardiac dynamics can be traced back to cellular events regulating intracellular Ca\textsuperscript{2+} homeostasis and, as such, provides an important conceptual framework from which students can begin to think about whole organ physiology in health and disease. Greater synchrony of Ca\textsuperscript{2+}-regulatory mechanisms between ventricular and pacemaker cells should enhance student comprehension of complex regulatory phenomenon in cardiac muscle.

the constant stream of new ideas and information related to the cellular regulation of cardiac contractile performance presents significant challenges with regard to identifying necessary conceptual information included for an introductory block of cardiovascular lectures at the graduate level. Often, we are limited to six to eight 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 emergent findings. The ongoing challenge for instructors is to provide graduate students with fundamental concepts and classical information while still providing exposure to new ideas. To this end, many have adopted a combined approach of using the cardiovascular learning objectives associated with Americal Physiological Society (APS) Medical Physiology Curriculum Objectives (http://www.the-aps.org/education/MedPhysObj/) in conjunction with supplementing assigned textbook readings with recent comprehensive review articles on cardiac excitation-contraction (E-C) coupling concepts. Another interesting approach we have adopted is the use of “classic papers” that can represent findings that are decades old or more contemporary classic papers such as original experiments related to the discovery of Ca\textsuperscript{2+} sparks (3). This approach has allowed for the prioritization of necessary core concepts while assuring exposure to evolving information to expand currently accepted dogma on traditional cardiovascular theory. The information contained herein is limited to key teaching concepts that should be considered for inclusion in a six- to eight-lecture block on the regulation of cardiac performance. Conceptual strategies and overarching themes for teaching traditional pump function within the context of myocyte cellular dynamics are included as well as some recent evidence that supports a paradigm shift in the basic regulation of cardiac automaticity. The slide presentation associated with this material can be found on the APS Education website (http://www.the-aps.org/education/refresher/CVPhysiology.htm), and the reader is referred to past Refresher Course articles on the teaching of traditional pump regulation and related physiology previously published in Advances in Physiological Education (4, 7). Here, three main take-home points are emphasized for a cardiovascular block of lectures: 1) the heart generates pressure and pressure makes the blood go round the closed loop circuitry, 2) mechanical events associated with one cardiac cycle can be traced back to Ca\textsuperscript{2+} movements and local control of E-C coupling, and an emerging theme that 3) local control of Ca\textsuperscript{2+} may also be responsible for the regulation of the cardiac pacemaker system.

Conversation Starter: the Fick Equation

A prevailing theme throughout my own lectures from which I never waiver 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\textsuperscript{2+} and/or myofilament Ca\textsuperscript{2+} sensitivity will alter left ventricular (LV) developed force. Thus, control of cardiac function ultimately occurs at the cellular level and allows for seamless transitions to topics such as the pharmacological regulation of LV function under pathological conditions. Important points of emphasis include the elegant design of the cardiovascular system, focusing on a “forest” view including the consequences of low venous versus high arterial pressures and differences in cardiac chamber pressures on blood flow as well as the necessary impact on vascular structure and function. Ultimately, the relationship among pressure, flow, and vascular resistance (characterized as a mathematical equation, where flow = (pressure/resistance)) is a key teaching concept to assist with integrating specific regulatory aspects of the arterial and venous circulations and predicting system behavior. The impact of parallel and series arrangements of the circulations becomes immediately apparent and provides an important segway to understanding vascular control mechanisms. In this regard, use of the Fick Equation for whole body O\textsubscript{2} consumption, where O\textsubscript{2} consumption = cardiac output ×...
arteriovenous O₂ difference and cardiac output = heart rate × stroke volume, also provides for important regulatory integrative processing. 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 stroke volume by intrinsic and extrinsic mechanisms. By providing this scheme at the outset, it becomes easier to transition back to the role of transsarcolemmal Ca²⁺ fluxes as a fundamental component to underlying cardiac receptor signaling, pharmacology, and functional responses in health and disease. Major intrinsic influences on cardiac pump function emphasized include the Frank-Starling effect and length-dependent regulation of stroke volume as well the treppe or force-frequency effect (details not presented here). Again, a reductionist approach allows for the compartmentalization of intrinsic and extrinsic regulatory mechanisms of both cardiac pump function as well as the regulation of heart rate.

**Microdomains and Local Control of E-C Coupling**

Central dogma of E-C coupling. For the purposes of this brief review, Fig. 1A shows the cellular events that underlie a single heart beat and shows phenomena related to global transsarcolemmal Ca²⁺ fluxes within a single ventricular myocyte. The central dogma underlying cardiac E-C coupling includes the theory of Ca²⁺-induced Ca²⁺ release (CICR), whereby small increases in Ca²⁺ in the vicinity of the sarcoplasmic reticulum (SR) lead to a much larger Ca²⁺ release from internal stores (SR). Upon membrane depolarization, Ca²⁺ enters the cytosol primarily through voltage-gated L-type Ca²⁺ current ($I_{Ca}$), which triggers Ca²⁺ release from the SR via Ca²⁺-release channels [ryanodine receptor isoform 2 (RyR2)]. Ca²⁺ is then free to bind to troponin C and engage the contractile machinery. We also know that small amounts of Ca²⁺ can enter the cell through reverse-mode Na⁺/Ca²⁺ exchanger (NCX) activity.

The diagram shown in Fig. 1B can also be used to illustrate the point that Ca²⁺ is required to activate the myofilaments and does so in a cooperative manner. Again, I stress the point that mechanisms that alter cytosolic Ca²⁺ and/or myofilament Ca²⁺ sensitivity alter developed force. This concept helps lay the foundation for the adrenergic regulation of cardiac contraction because it is known that voltage-gated Ca²⁺ channels (VGCC), RyRs, phospholamban, and troponin I can all be phosphorylated by PKA subsequent to β₂-adrenergic activation (see below and Ref. 1). Additional kinases, such as Ca²⁺/calmodulin-dependent kinase II (CaMKII), are also known to regulate E-C coupling regulatory proteins subsequent to PKA activation or via PKA-independent effects (for a review, see Ref. 1). In this regard, calmodulin can exert direct effects on membrane ion channels such as VGCCs.

Ca²⁺ sparks underlie the Ca²⁺ transient (intracellular Ca²⁺ concentration). A commonly accepted topic for lecture inclusion includes the notion that Ca²⁺ sparks underlie the intracellular Ca²⁺ concentration ([Ca²⁺]), in their landmark report, Cheng and colleagues (3) identified Ca²⁺ sparks as the elementary events underlying SR Ca²⁺ release in ventricular myocytes, thereby providing support for the local control theory of cardiac E-C coupling. Thus, an important point of emphasis to students is that while the [Ca²⁺], that controls contraction is largely a global process (cell wide), as shown in Fig. 1, Ca²⁺ release from the SR is largely controlled at the local (subcellular) level. Importantly, normal E-C coupling involves a well-ordered and stereotyped sequence of events that includes Ca²⁺ sparks, and Ca²⁺ sparks are currently thought to be the fundamental units of SR Ca²⁺ release occurring both at rest and during contractile activation. Important points of emphasis for Ca²⁺ sparks and the local control theory include the following: 1) summation of Ca²⁺ sparks provides the microscopic basis of [Ca²⁺]; 2) Ca²⁺ sparks provide an explanation for graded contractions, which are modulated by local control; 3) Ca²⁺ sparks provide insights into microdomains and where they occur; and 4) Ca²⁺ sparks

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**Fig. 1.** Cardiac excitation-contraction coupling events. A: electrical excitation at the sarcolemmal membrane activates voltage-gated Ca²⁺ channels, and the resulting Ca²⁺ entry activates Ca²⁺ 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 Ca²⁺ influx mechanisms and those above the line are processes related to Ca²⁺ efflux. For relaxation to occur, the intracellular Ca²⁺ concentration must decline, allowing Ca²⁺ to dissociate from troponin. This involves the transport of Ca²⁺ out the cytosol by one of four pathways: the SR Ca²⁺-ATPase pump located on the SR [sarco(endo)plasmic reticulum Ca²⁺-ATPase (SERCA)], a sarcolemmal Na⁺/Ca²⁺ exchanger (NCX), a sarcolemmal Ca²⁺-ATPase, and a mitochondrial uniporter. The SERCA pump is quantitatively the most important cellular mechanism for Ca²⁺ removal and, hence, relaxation, and, depending on the species of interest, can account for ~70% of Ca²⁺ removal, whereas NCX accounts for ~28% of Ca²⁺ removal. PLB, phospholamban; Mito, mitochondria. B: this diagram can also be used to illustrate the point that Ca²⁺ is required to activate the myofilaments and does so in a cooperative manner. Δ[Ca²⁺]local change in total Ca²⁺ concentration. [Modified from Bers (2) with permission.]
provide insights into pathological changes in E-C coupling such as Ca\(^{2+}\) waves and cardiac arrhythmias. Given emerging evidence that local Ca\(^{2+}\) signaling is critical to cardiac autonomicity (described in detail below), emphasis on the local control theory also lays the foundation for its role in sinoatrial nodal cell (SANC) regulation.

Regulation of the Inotropic State: Intrinsic Versus Extrinsic β-Adrenergic Signaling

Since Frank-Starling and force-frequency effects are fairly well characterized in most textbooks, the primary focus of this section is to briefly review the mechanisms of adrenergic regulation of the inotropic state. Well-known effects of sympathetic nervous system stimulation on cardiac β₁-adrenergic receptors (ARs) include both enhanced contraction and relaxation mediated by PKA phosphorylation of VGCCs, RyRs, troponin I, and phospholamban, respectively, and subsequent effects on intracellular Ca\(^{2+}\) homeostasis. However, the contribution of additional G protein-coupled receptors (GPCRs) as well as mediators released by the endothelium, such as nitric oxide, that modulate muscle function and associated cardiac contraction may assume increasing importance under a variety of clinical circumstances. In this regard, use of pressure-volume loops to illustrate true changes in cardiac contractility independent of changes in preload and afterload helps to emphasize the importance of extrinsic regulators of cardiac performance.

Signal transduction and myocardial performance. The β₁-AR is clearly the predominant GPCR in the heart regulating positive inotropic effects. The β₁-AR is coupled to G\(_\text{s}\), and results in the activation of PKA. However, it is important to recognize additional GPCRs known to be present in the heart, such as β₂-ARs and α₁-ARs, exert varying degrees of cardiac inotropy (6). Activation of β₂-ARs results in dual coupling to both stimulatory and inhibitory G proteins (G\(_\text{s}\) and G\(_\text{i}\)) and represents an elegant example of how redundancy in cellular signaling can simultaneously initiate and terminate an activator signal and allow for the fine tuning of LV contractile performance. The α₁-AR is coupled to G\(_\text{q}\) as are the GPCRs for angiotensin II and endothelin, which result in the production of the second messengers inositol triphosphate and diacylglycerol as well as the 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. Since the students are equally familiar with cardiac E-C coupling, effects of receptor stimulation on E-C coupling dynamics and Ca\(^{2+}\) homeostasis become evident.

Desensitization and protein scaffolds. 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 the activation of G protein-coupled receptor kinase 2 (GRK2; also known as β-AR kinase 1), whereby GRK2 phosphorylates agonist-occupied receptors. 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 β₁-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 the activation of ERK signal transduction pathways. This dual signaling sheds light on possible mechanisms of receptor cross-talk and, in the case of the heart, provides insights into how one receptor, such as the α₁-AR, can induce both inotropic and growth effects, respectively.

What Is the Role of Local Ca\(^{2+}\) Cycling in Cardiac Pacemaker Function and Intrinsic Regulation of Heart Rate?

A paradigm shift in teaching cardiac autonomicity and regulation of the heart rate: role of the coupled-clock pacemaker system and Ca\(^{2+}\) cycling. A concept worthy of incorporation into lectures on cardiac autonomicity includes some degree of focus on an emerging concept involving the role of the “coupled-clock pacemaker system” and local Ca\(^{2+}\) signaling in SANC regulation (for a recent review, see Ref. 5). The details of this regulation continue to evolve; however, the notion that sequestration and release of Ca\(^{2+}\) from internal stores in SANCs (similar to that observed in ventricular myocytes described above) regulates rhythmic cardiac excitation (i.e., membrane ion channels) is an important advancement in this area.

Central dogma of automaticity. A unique property of cardiac muscle includes automaticity, a function that is primarily associated with SANCs located in the right atrium. This anatomic location is commonly referred to as the natural pacemaker of the heart and represents the site of origin of the electrical signal that subsequently allows for the depolarization of cardiac muscle and intrinsic rhythmicity. The intrinsic rhythmicity of the sinoatrial node is, in turn, thought to be controlled by three time- and voltage-dependent membrane currents: I\(_\text{Ca-L}\), outward K\(^+\) current (I\(_\text{K}_{\text{ass}}\)), and nonspecific cation “funny” current (I\(_\text{f}\)). Current dogma suggests that the ionic basis of automaticity and the characteristic slow diastolic depolarization (DD) unique to SANCs is primarily due to activation of I\(_\text{f}\), which is activated at the end of phase 3 of the action potential with a threshold for activation of approximately −40 mV (Fig. 2). The hyperpolarization-activated, cyclic nucleotide-gated (HCN) channel is thought to underlie I\(_\text{f}\) and is a nonspecific cation channel that conducts both Na\(^+\) and K\(^+\) and, as mentioned previously, becomes activated during the repolarization phase of the action potential. Ca\(^{2+}\) influx via I\(_\text{Ca-L}\) accelerates the rate of DD, leading to the upstroke of the action potential, whereas efflux of K\(^+\) and activation of I\(_\text{K}_{\text{ass}}\) contributes to SANC repolarization. Importantly, SANCs are under the tonic influence of the parasympathetic nervous system in the basal state, slowing the heart rate to reflect an average resting heart rate of 60–70 beats/min in humans.

Negative chronotropic effects mediated by parasympathetic influence and the subsequent release of ACH from the vagus nerve slows the intrinsic firing rate of the pacemaker by three proposed mechanisms: 1) decreasing I\(_\text{f}\), 2) increasing K\(^+\) conductance and making the maximum diastolic potential more negative (inhibitory effects on K\(^+\) channels mediated by the Gβγ subunit of the ACH GPCR), and 3) inhibition of adenyl cyclase and reductions in I\(_\text{Ca-L}\) and steepness of phase 4 depolarization. In contrast, sympathetic influence increases heart rate...
rate through β₁-AR activation by two primary mechanisms: 1) increasing \(I_f\), thereby increasing the steepness of phase 4; and 2) increasing \(I_{Ca}\), thus shifting the depolarization threshold to more negative values.

The coupled-clock pacemaker system. In this new scheme, surface membrane ion channels, as described above (see also Fig. 3), remain an integral part of the automaticity paradigm to function as a “membrane” (M) clock. However, a major tenet of the coupled-clock pacemaker system suggests that rhythmic RyR-generated subsarcolemmal local \(Ca^{2+}\) releases (LCRs) function as a “Ca^{2+} clock,” which ultimately underlies the pacemaker function of SANCs, leading to the mutual entrainment of Ca^{2+} and M clocks to regulate automaticity (Fig. 3). Specifically, LCRs occur during DD and activate inward NCX current (\(I_{NCX}\)), which represents a key step in the coupled-clock system. LCRs accelerate late DD, and the influx of Ca^{2+} drives the membrane potential to threshold through \(I_{Ca}\) opening, which induces local CICR (i.e., SR Ca^{2+} release from RyRs) and the rapid upstroke. The action potential-induced global CICR results in synchronized SR Ca^{2+} depletion. SR Ca^{2+} depletion represents another critical step in the coupled-clock system, which terminates the LCRs and resets the Ca^{2+} clock by rendering RyRs inactive. Importantly, Ca^{2+} influx via \(I_{Ca}\) during depolarization also serves to refill SR Ca^{2+} stores.

![Fig. 2. Characteristic cardiac sinoatrial node action potential. The intrinsic rhythmicity of the sinoatrial node is thought to be controlled by three time- and voltage-dependent membrane currents: inward L-type Ca^{2+} current (\(I_{Ca}\)), outward K⁺ current (\(I_K\)), and nonspecific cation “funny” current (\(I_f\)).](http://advan.physiology.org/)

![Fig. 3. Key phases of the coupled-clock pacemaker system. CICR, Ca^{2+}-induced Ca^{2+} release; DD, diastolic depolarization; \(I_{CaL}\), inward L-type Ca^{2+} channel current; \(I_{CaT}\), T-type Ca^{2+} channel current; \(I_{NCX}\), NCX current; MDP, maximum diastolic potential. [Reprinted from Lakatta (5) with permission.]](http://advan.physiology.org/)
providing for the constancy of SR Ca\(^{2+}\) load on a beat-to-beat basis and ensuring proper Ca\(^{2+}\) clock operation. Activation of \(I_{\text{K}}\) (through \(I_{\text{Ca}}\) and \([\text{Ca}^{2+}]_{i}\)) contributes to repolarization and Ca\(^{2+}\) efflux (early DD). Importantly, without the LCR-activated \(I_{\text{KCCX}}\) "prompt" to the membrane, SANC depolarization does not occur.

Functional interactions occur between the Ca\(^{2+}\) and M clocks and are critical for normal automaticity (hence, the name "coupled-clock pacemaker system"). The LCR period, defined as the time between the onset of global SR Ca\(^{2+}\) depletion triggered by the action potential and the spontaneous emergence of LCRs during the subsequent DD (Fig. 3), is a key regulated variable that determines the "ticking speed" of the coupled-clock system. In addition to Ca\(^{2+}\), basal protein phosphorylations affect the function of both M and Ca\(^{2+}\) clock proteins, which are critical to the regulation of normal automaticity (Fig. 4). Specifically, Ca\(^{2+}\)-activated CaMKII and cAMP-mediated, PKA-dependent phosphorylation of Ca\(^{2+}\) cycling and membrane proteins (as described above for ventricular muscle) occur in the basal state and are required for normal operation of the system. High constitutive activation of Ca\(^{2+}\)-activated adenyl cyclase types 1 and 8 contribute to PKA phosphorylations (VGCCs, K\(^{+}\) channels, RyRs, and phospholamban) and, combined with CaMKII activation [VGCCs, RyRs, phospholamban, and sarco(endo)plasmic reticulum Ca\(^{2+}\)-ATPase], regulate the cell Ca\(^{2+}\) balance and SR Ca\(^{2+}\) cycling (see also Fig. 4). High constitutive phosphodiesterase and protein phosphatase activities in SANCs serve as important control points to keep Ca\(^{2+}\) signaling in balance. In this model, the contribution of \(I_{f}\) is controversial, but evidence has suggested that the HCN channel may also be activated by high basal levels of cAMP and contribute to heart rate slowing.

Pacemaker rate modulation by cholinergic and adrenergic signaling. As described for cardiac inotropic effects, GPCR signaling can also modulate a number of membrane and Ca\(^{2+}\)-cycling proteins in SANCs that influence the LCR period and cycle length and, hence, modulate pacemaker ticking speed, i.e., heart rate. Sympathetic stimulation and subsequent activation of the \(\beta_{1}\)-AR reduces the LCR period, shifting LCR occurrence to an earlier time during the DD by increasing cAMP, PKA, and CaMKII-dependent phosphorylations of sarcolemmal and Ca\(^{2+}\)-cycling proteins, as described above (see also Fig. 4). In contrast, prolongation of the LCR period is induced by cholinergic stimulation. ACh receptor stimulation is known to suppress LCRs through the activation of Gi and therefore contributes substantially to slowing of the heart rate primarily through effects on K\(^{+}\) channel activation and hastening repolarization. Collectively, phosphorylation of proteins that regulate Ca\(^{2+}\) within the coupled-clock system leads to alterations in the LCR period by GPCR signaling, cause graded changes in the timing of the onset and amplitude of the late DD exponential increase, and thus changes to the steady-state action potential cycle length. Indirect effects on Ca\(^{2+}\) cycling will also influence action potential cycle length, as described above.

**Summary**

Use of an integrative "systems" pedagogical approach provides a unique opportunity to emphasize a forest view of cardiac regulatory dynamics while at the same time providing a context for emphasizing specific cellular details, tracing key mechanical events back to subsarcolemmal Ca\(^{2+}\) regulation. Recent developments in SANC automaticity theory have led to greater synchrony of Ca\(^{2+}\)-regulatory mechanisms between ventricular and atrial myocyte regulation, respectively, which should enhance student comprehension of complex regulatory phenomenon. Specifically, cardiac rate regulation governed by intracellular Ca\(^{2+}\) cycling and by coupling to surface membrane proteins represent the clocks that control SANC automaticity. The rate and amplitude of SR Ca\(^{2+}\) cycling are

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**Fig. 4.** Regulatory interactions between the membrane (M) clock and Ca\(^{2+}\) clock. \(I_{\text{KAC-NAC}}\): ACh-activated K\(^{+}\) current; A cyclase, adenylyl cyclase; HCN, hyperpolarization-activated, cyclic nucleotide-gated channel; \(I_{\text{Ks}}\): slow component of the delayed rectifier K\(^{+}\) current; GPCR, G protein-coupled receptor; CaM, calmodulin; CaMK-II, Ca\(^{2+}\)/calmodulin-dependent kinase II. [Reprinted from Lakatta (5) with permission.]
controlled by the amount of free Ca\(^{2+}\) in the system, the SR pumping rate, and the number of activated RyRs. The LCR period and amplitude determine the time and amplitude of the late exponential DD phase and, thus, whether the membrane achieves excitation threshold to generate the next rhythmic action potential via activation of \(I_{\text{NCX}}\). Pacemaker rate regulation is ensured by coupling SR Ca\(^{2+}\) cycling to surface membrane GPCR signaling.

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