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Compartmentation of membrane processes and nucleotide dynamics in diffusion-restricted cardiac cell microenvironment

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Abstract

Orchestrated excitation–contraction coupling in heart muscle requires adequate spatial arrangement of systems responsible for ion movement and metabolite turnover. Co-localization of regulatory and transporting proteins into macromolecular complexes within an environment of microanatomical cell components raises intracellular diffusion barriers that hamper the mobility of metabolites and signaling molecules. Compared to substrate diffusion in the cytosol, diffusional restrictions underneath the sarcolemma are much larger and could impede ion and nucleotide movement by a factor of 10^3–10^5. Diffusion barriers thus seclude metabolites within the submembrane space enabling rapid and vectorial effector targeting, yet hinder energy supply from the bulk cytosolic space implicating the necessity for a shunting transfer mechanism. Here, we address principles of membrane protein compartmentation, phosphotransfer enzyme-facilitated interdomain energy transfer, and nucleotide signal dynamics at the subsarcolemma–cytosol interface. This article is part of a Special Issue entitled 'Local Signaling in Myocytes'.

Keywords

ADP; ATP; ATP-sensitive K^+ channel; Energy metabolism; Ion homeostasis; Macromolecular complex; Partitioning; Phosphotransfer; Submembrane

1. Introduction

Established in Ancient Rome, the strategic principle *divide et impera* (divide and conquer) implies the fragmentation of a large concentration of power into pieces that individually can be more readily defeated and then managed allowing to gain and maintain control on a

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Disclosure statement

None.

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whole dominion. In biology, the evolutionary advantage of eukaryotes over prokaryotes is signified by intracellular partitioning of biological processes enabling more efficient and better controlled cellular and ultimately body metabolic well-being [1,2]. Localization of metabolic pathways within organelles enables eukaryotic cells to concomitantly carry out distinct metabolic activities. To this end, compartmentation ensures a targeted hierarchy that: i) establishes physical boundaries for metabolic reactions; ii) creates microenvironments for spatial/temporal regulation of biological processes; and iii) secures vectors of cell response matching environmental signaling—all through functionally specialized cellular spaces and specifically positioned enzymes and substrates within organelle compartments [3]. With compartmentation, environmental signaling and feed-back rectification of cellular metabolic balance dictate the operation of molecular effectors, such as ion channels, in a milieu of physical barriers imposed by biomembranes and structural protein complexes [4]. A case in point is the high-fidelity orchestration of cardiac muscle function, which requires a fine-tuned system of inter-compartment communication for proper operation, exemplified by movement of ions and energy substrates in excitation–contraction coupling. Recent evidence suggest that bringing signaling proteins and ion channels into spatial proximity, in a not well-mixed cytoplasm, is an essential principle of how diverse cellular targets can decipher and translate promiscuous messengers, e.g., intracellular Ca^{2+} or cyclic nucleotides, into selective responses. Thus assessing the principles and properties of compartmentation of ion channels, metabolites and energy pathways in a partitioned cell environment expands current understanding of heart homeostasis maintenance.

2. Ion channels in compartmentalized membrane macromolecular complexes

A relevant question in cell signaling, and in particular cardiac physiology, is how a limited variety of intracellular second messengers can differentially relay environmental signals associated with a large number of surface neurotransmitter or hormone receptors to effectively regulate electrical, mechanical and metabolic activity [5–8]. Indeed, many membrane receptors interact with heteromeric G-proteins in response to ligand binding [9]. Dissociation of G-proteins into Gα and Gβγ subunits initiates the production of second messengers (e.g., cAMP) and propagation of signals within the cell resulting in altered activity of different downstream effector proteins, such as enzymes and ion channels [10]. The rationale has emerged that cells ensure specificity of signaling pathways through organized macromolecular signaling complexes in the plasma membrane that may contain critical transducers, e.g., adenylate cyclase, protein kinase A (PKA) and PP2A phosphatase, as well as L-type Ca^{2+} channels all coupled to G-protein receptors [11,12]. Assuming that components of macromolecular complexes are physically anchored in the membrane vicinity, rapid and specific activation of the correct signaling pathway can be ensured. Such role for PKA anchoring proteins (AKAPs) is underscored by a specific binding motif to regulatory subunits (RII) serving as an adapter that maintains the holoenzyme at specific subcellular locations (Fig. 1) [13].

An alternative mechanism of protein clustering has been associated with the anchoring role of cytoskeletal proteins, such as spectrins and ankyrins, which line the intracellular side of the plasma membrane [14–16]. Specifically, ankyrin-G and β_{IV}-spectrin are critical structural components required for clustering of voltage-gated Na^+ channels ($Na_v1.5$) [17–19] and calcium/calmodulin-dependent protein kinase II (CaMKII) in cardiac cells [20,21]. Disruption of β_{IV}-spectrin-dependent co-localization of CaMKII and $Na_v1.5$ results in lack of phosphorylated Na^+ channels and aberrant cell excitability [21]. High-resolution imaging, using a glass micropipette as a probe in scanning ion conductance microscopy [22,23], has revealed the position of ATP-sensitive potassium (K_{ATP}) channels in the Z-grooves of

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cardiac myocytes [24], sarcolemmal regions that interact with the intracellular cytoskeleton and where transverse tubules (T-tubules) create membrane junctions with the sarcoplasmic reticulum (SR) [25]. In the same region, voltage-gated Na^+ channels along with the IP_3 receptor, Na^+-Ca^{2+} exchanger and Na^+/K^+ ATPase were identified, and all, including K_{ATP} channels, found to be coordinated through the membrane-binding domains of ankyrins to the spectrin-actin based membrane cytoskeleton (Fig. 2) [26–29]. Such compartmentation is efficient at coordination of essential components that control excitation–contraction coupling in a manner that is rapid, yet precise. Readiness for a prompt response is supported by voltage-gated Ca^{2+} (CaV) channels distributed throughout T-tubules [25,30]. Conformational rearrangements of CaV proteins in response to changes in membrane potential are translated directly to ryanodine receptors (RyR) initiating Ca^{2+} release from the sarcoplasmic reticulum [31]. Indeed, the so-called Calcium Release Units (CRU), a group of Ca^{2+} permeant RyR or IP_3R channels clustered in the membrane of the sarcoplasmic reticulum, are responsible for the elementary intracellular Ca^{2+} release events (calcium sparks) that compartmentalize to the Z-line/T-tubule regions of sarcomeres [32–34]. Thus, the local ensemble of channel proteins directly regulating membrane excitability, along with exchangers and pumps aimed at ion sequestration supporting ion homeostasis, ensure rapid and near simultaneous release of Ca^{2+} to bind adjacent troponin molecules and enable high-fidelity regulation of myosin–actin contraction (Figs. 1 and 2) [12].

A remarkable mechanism of ion channel localization appears to be the dynamic compartmentation between the endoplasmic reticulum (ER) and voltage-independent Ca^{2+} channels responsible for store-operated Ca^{2+} entry (SOCE), also known as Ca^{2+}-release-activated Ca^{2+} (CRAC) current identified in non-excitable cells [12,35] as well as embryonic and neonatal cardiac myocytes [36]. While Ca^{2+} release from ER in response to the phospholipase C (PLC) product, inositol-1,4,5-trisphosphate (IP_3), triggers transient intracellular Ca^{2+} elevation, the mechanism of sustained subsequent Ca^{2+} entry is largely unknown [35,37]. The hallmark observation that slow, over seconds, activation of SOCE through plasma membrane was not initiated by a rise in cytoplasmic Ca^{2+} but rather started following depletion of Ca^{2+} in the endoplasmic reticulum stores [38], suggested an intimate interaction between unidentified components of ER and CRAC channels. Discovery of the stromal interaction molecule 1 (STIM1) as an ER Ca^{2+} sensor, and Orai1 as a functional component of CRAC channels, were key steps in defining the mechanism of SOCE [39,40]. Depletion of Ca^{2+} stores is sensed by STIM1, resulting in accumulation in ER regions adjacent to the plasma membrane. In turn, Orai1 molecules move from a dispersed distribution in plasma membrane to be assembled directly opposite to STIM1 clusters, presumably compartmentalizing both proteins in close proximity and enabling STIM1 to activate CRAC channels [41,42].

3. Diffusion restriction—new dimension in cell signaling

To ensure effective and precise control over cellular functions, spatial distribution of individual membrane components, which facilitates their recognition and interaction, has to be accompanied by an additional dimension of intracellular compartmentation. Analysis of the involvement of the Na^+-Ca^{2+} exchanger in cardiac excitation–contraction coupling suggested a unique component of cardiomyocyte architecture, namely a diffusion barrier underneath the sarcolemma that secludes a so-called "fuzzy space" from the rest of the cell [43]. The Na^+–Ca^{2+} exchanger may support Ca^{2+} influx by "pumping" internal Na^+ out, or Ca^{2+} efflux by "pumping" external Na^+ into the cell in a stoichiometry of 3 Na^+ to 1 Ca^{2+} depending on the thermodynamic driving force and electrochemical gradient for Na^+ ions [44]. In a ventricular myocyte with dimensions 10 by 10 by 100 μm, volume 10^{-11} l and capacitance 50 pF, depolarization from −80 mV to +20 mV would require a charge translocation of ~5×10^{-12} Coulomb (30×10^6 ions) that can increase Na^+ concentration in

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the whole cell volume by only 5 μM above normal intracellular Na^+ levels of ~8 mM. This average cytosolic Na^+ concentration is insufficient to activate Na^+–Ca^{2+} exchange due to the much higher value of the apparent dissociation constant for Na^+ estimated at ~20 mM [45]. Therefore the volume into which entering Na^+ ions accumulate must be significantly restricted. If such diffusion-restricted space is limited to 100–200 nm below the inner surface of the sarcolemma (0.02–0.04% of total cell volume), a 2500–5000-fold elevation of Na^+ concentration would be then sufficient to rise Ca^{2+} levels in this hypothetical volume through the Na^+–Ca^{2+} exchanger between 1 and 10 μM/ms contributing, thereby, to excitation–contraction coupling [43,45]. In subsequent studies the apparent diffusion coefficient for Na^+ ions in the "fuzzy space" was estimated to be 10^3 to 10^4-fold below that expected in bulk cytoplasm [46].

An analogous approach that implemented ion channels as prototypic membrane sensors allowed understanding of the spatial and temporal nature of cAMP as well as ATP/ADP signaling [47–51]. Having established nucleotide-dependent regulation of cyclic nucleotide-gated (CNG) and ATP-sensitive K^+ (K_{ATP}) channels, the local concentration of nucleotides in the plasma membrane proximity, where channels reside, could also be determined [52–56].

Evidence obtained using cyclic nucleotide-sensing CNG channels indicate the presence of significant diffusional limitations for cAMP near the inner surface of the plasma membrane [57]. Specifically, the operation of CNG channels indicated that submembrane elevation of cAMP in response to activation of adenylyl cyclase (AC) was approximately 12-fold higher than throughout the cell [52]. Co-localization of CNG channels and AC would be insufficient to explain this difference since in the absence of a diffusional barrier each newly synthesized cAMP molecule would diffuse away from the sensor faster than the next one can be produced, provided that the diffusion coefficient for cAMP is 3×10^{-6} cm^2/s (the coefficient for free diffusion of cAMP in cytosol) [57]. Analysis of cAMP flux from the submembrane to the cytosolic space revealed that the local concentration of cAMP measured by CNG channels required that diffusion within the 200 nm near membrane "fuzzy space" must be limited by an apparent diffusion coefficient of ~10^{-13} cm^2/s [52,57], indicating that the submembrane compartment is an unstirred zone with extremely restricted nucleotide diffusion.

Notably, an alternative and independent analysis of nucleotide fluxes in the submembrane space, performed with K_{ATP} channels, provides close estimates for diffusion restrictions [49,53,55]. In contrast to CNG channels that are activated by a rise in cAMP, K_{ATP} channels, by virtue of tight coupling with cellular energetics, open in response to a drop of intracellular ATP and a concomitant increase of ADP levels [50,58–60]. As cytosolic ATP levels are well maintained by intracellular energetics and K_{ATP} channel activity is poorly correlated with changes in total ATP/ADP [61–63], it has been proposed that sarcolemma-associated ATPases depress local ATP concentration setting a nucleotide ratio at the channel site, within the diffusion-restricted submembrane space, distinct from cytosolic levels [49,55,58,59,61,64,65]. This notion is in accord with the finding that K_{ATP} channels complement membrane macromolecular complexes that also include Na^+ channels, Na^+/K^+ ATPase and Na^+–Ca^{2+} exchangers, and are co-localized with Ca^{2+} channels and Ca^{2+}-scavenging ATPases [24,26,27,66,67]. Thus, compartmentalized K_{ATP} channels appear to function in a microenvironment where local nucleotide content is distinct from average cytosolic levels [4,50]. Indeed, application of 20–40 mM of Na^+ to permeabilized cardiac myocytes [49] or to giant excised patches [53] produced ouabain-sensitive K_{ATP} channel openings, as a result of elevated Na^+/K^+ ATPase activity, despite the inhibitory bulk ATP concentration. Even in excised giant patches, where plasma membrane itself and structures adjacent to the sarcolemma could be significantly disturbed, the Na^+/K^+ ATPase-driven

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depletion of ATP, detected by K_{ATP} channels within the 200 nm submembrane space, can be explained by an apparent diffusion coefficient of 7×10^{-10} cm^2/s [53], much lower than ~10^{-6} cm^2/s, the coefficient of free diffusion for nucleotides in the cytosol [68].

In cardiac cells, myoplasmic ATP under normal (aerobic) conditions is estimated at ~6–10 mM, and under severe metabolic poisoning by cyanide total ATP levels drop to 80% of control levels [61,65,69]. This drop is insufficient to explain K_{ATP} channel-driven shortening of action potentials in response to chemical ischemia. Nucleotide-dependent K_{ATP} channel gating indicates that for MgADP to open at least 1% of K_{ATP} channels, which has been suggested to be sufficient to elicit detectable action potential shortening [58], ATP at the channel site needs to be reduced to <3 mM [55]. Assuming an average bulk intracellular ATP concentration of 7 mM, a sarcolemmal ATPase flux (J_{ATPase}) of 4.7×10^{-6} μmol/cm^2/s, estimated in working hearts based on ^{18}O-asssited ^{31}P NMR [55,70], can induce a 4 mM drop of ATP levels only at an apparent diffusion coefficient of 2.3×10^{-11} cm^2/s within a 200 nm submembrane space [55]. Adding to consideration a local adenylate kinase activity (2·ADP ↔ ATP + AMP), which can support submembrane ATP from ADP produced by local ATPase activity, the diffusion coefficient must be further reduced to 1.6×10^{-11} cm^2/s [49]. Although the value of restricted nucleotide diffusion has been obtained with certain assumptions, a five orders of magnitude difference from the diffusion coefficient in the cytosol cannot be compensated through realistic variations of submembrane space thickness, ATPase flux, or bulk ATP.

A number of observations do indicate the existence of such a surprising barrier secluding the unstirred layer near the plasma membrane. The physical nature of the reduced metabolite mobility near the sarcolemma may result from protein aggregates [26,71], membrane surface positive charges [72], reticular membranes and other organelles [4], and the underlying barrier should be considered as a significant contributor to intracellular signaling. The diffusion barrier differentiates a transient reaction of submembrane effectors to local accumulation of cAMP from a sustained response of cytosolic effectors to cAMP signaling [47,51,52]. Further evidence for the role of submembrane compartmentation in cardiac physiology was obtained in a heart failure model induced by transgenic expression of tumor necrosis factor [73,74]. Cardiac myocytes from transgenic failing hearts underwent significant distortion of cellular architecture (Fig. 3A) [75]. Cell remodeling disturbed energy communication between the cytosol and the submembrane space revealed by sarcolemmal K_{ATP} channel recordings. While in excised membrane patches K_{ATP} channels displayed normal biophysical and regulatory properties, at both cellular and organ levels K_{ATP} channels demonstrated a blunted response to chemical hypoxia due to disrupted energy communication between cell compartments (Figs. 3B and C) [75]. Intracellular structural rearrangements that prevented prompt K_{ATP} channel openings following onset of DNP-induced metabolic inhibition could have also set an environment that impeded efflux of local glycolysis-produced ATP from submembrane space, restraining K_{ATP} channel activity [76–78]. Thus, in the setting of cardiomyopathy, spatial remodeling including disturbances in submembrane compartments is associated with compromised feed-back regulation of energy expenditure by K_{ATP} channels, secluded in the sarcolemma, in response to development of bulk energy deficit [49,50,75,79–82].

4. Energy communication in a compartmentalized cellular environment

The modern concept that failing hearts are engines out of fuel has emerged with recognition that altered energetics precipitate organ failure [81,83–85]. In cellular compartmentalized environments, where metabolite mobility between microdomains is limited, unregulated local ATP utilization, in the absence of mechanisms capable of shunting diffusional barriers, may significantly deplete local energy stores generating energy deficient phenotypes

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[50,86]. The earlier concept implying that ATP diffuses freely in an isotropic cell milieu considered as a “well-mixed bag of enzymes” [87], no longer explains ATP compartmentation in myofibrils, which could result in a paradoxical energy deficit in cardiac cells under conditions of high and well-preserved average ATP levels [4,79,88]. Diffusion restrictions in the submembrane space would hamper communication between bulk and submembrane domains, restricting membrane processes to function within local fluctuations of metabolites. While adenine nucleotides (ATP, ADP) are the recognized cellular energetic currency, the molecular mechanisms of energy signaling between compartments remain partially conceptualized.

Energy consumption is no longer viewed as a process whereby freely diffusing high-energy phosphates are consumed where needed throughout the cell. Several observations support the notion that energy consumption is also compartmentalized [4,71,79,89,90]. In the heart, selective inhibition of anaerobic glycolysis versus mitochondrial oxidative phosphorylation has revealed a differential efficacy of these systems in supporting specific membrane versus contractile functions, which cannot be explained by changes in total transcellular high-energy phosphate levels [76,77,91,92]. Anomalously slow ATP diffusion, as a result of macromolecular obstacles, was identified within sarcomeres, indicating that even under a physiological increase in cardiac workload, with an elevation of the rate of myosin ATPase from 3.4 to 5.2 mM/s, ATP concentration in the center of the sarcomeric A-zone rapidly disappears reaching 0.1 mM, which without effective energy transfer, may result in energy deficit, contractile dysfunction and cell damage [79]. Phosphotransfer enzymes creatine and adenylate kinases (CK and AK) have been recognized as systems that can facilitate energy transfer between sites of ATP production and utilization [84,93–98]. Widely distributed in cellular compartments, CK and AK are co-localized with energy sensing K_{ATP} channels [55,64,86,98–102] and thereby with cytoskeleton-bound sarcolemmal complexes [26,27], integrating into membrane metabolic units (Fig. 4A). As nucleotide-sensing K_{ATP} channels are co-localized within the metabolic unit with ion transporting ATPases, channel openings induced by a local drop of ATP and an increase of ADP levels can accelerate repolarization of action potentials by driving membrane potential towards the K^+ equilibrium restraining, thereby, associated energy expenses. Thus K_{ATP} channels, when necessary, can provide a feed-back response to limit processes occurring during action potential propagation, including the operational time of L-type Ca^{2+} channels and associated Ca^{2+} release from sarcoplasmic reticulum as well as the concomitant activity of the Na^+–Ca^{2+} exchanger, myosin-, Ca^{2+}- and Na^+/K^+-ATPases [50,58,103,104].

The reversible CK reaction, ADP + CrP↔Cr + ATP, phosphorylates ADP by transferring phosphate from creatine phosphate (CrP) producing creatine (Cr) catalyzing CrP-ATP phosphotransfer. The high value of the reaction equilibrium constant K_{CK}=160 [105] indicates that an augmentation of ATP hydrolysis depletes the intracellular pool of CrP to a greater extent than the pool of ATP. Such buffering could be interpreted as a result of facilitated delivery of ATP from one cellular compartment to another accompanied by diffusion of high-energy phosphate equivalents (CrP), which are then locally involved in CK-catalyzed ATP synthesis [49,50]. Indeed, changes in CK reaction flux tightly follow cellular energetic dynamics due to significant partaking of the CK system in energy communication between cell compartments [55,70,93].

Adenylate kinase (AK) can also facilitate nucleotide intercompartmental exchange by AMP diffusion flux (J_{AMP}) with an equilibrium constant K_{AK} = 1 [70,100,105,106]. Located in the mitochondria, cytosol, and membrane-bound [107,108] adenylate kinase has a distinct role in setting the cell response to stress through activation of AMP-dependent processes [109,110]. Gene deletion of creatine kinase or adenylate kinase isoforms generates

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phenotypes with increased electrical vulnerability, disturbed muscle energetic economy and decreased tolerance to metabolic stress [92,107].

Integration of the CK system with the energy network, assuming conditions of equilibrium, implies that membrane ATPase flux, in addition to ATP diffusion, is equalized by CK activity manifested by creatine phosphate influx (J_{CrP}) to submembrane or creatine efflux ($-J_{Cr}$) to bulk space (Fig. 4A). As AMP is a co-product of AK catalysis, the local AK reaction is marked by efflux of AMP from submembrane to bulk space ($-J_{AMP}$). Thus ATP consumption in the submembrane “fuzzy space” (J_{ATPase}) can be described through ATP, CrP and AMP diffusion fluxes as $J_{ATPase}=J_{ATP}+J_{CrP}-J_{AMP}$ (Fig. 4A) [49]. Resolving this equation using Fick's 1st law (assuming equilibrium conditions) with a diffusion coefficient value of 1.6×10^{-11} cm^2/s (defined above), ATPase flux of 4.7×10^{-6} μmol/cm^2/s, 7 mM total nucleotide bulk pool and a thickness of the submembrane compartment 200 nm [49,50] allowed the following prediction regarding phosphotransfer reactions setting nucleotide gradients between compartments (Fig. 4B): i) within a wide range of membrane ATPase activity, the cooperative action of CK and AK virtually nullifies differences between bulk and submembrane nucleotide concentrations ($\Delta ATP = [ATP]_b - [ATP]_m = 0$) at a high bulk ATP; ii) CK phosphotransfer is able to amplify cytosolic signals at a diffusion barrier, e.g., reduction by only 0.25 mM (from 6.85 to 6.6 mM) in cytosolic ATP generates a 2 mM ATP gradient between the cytosol and sarcolemma through the diffusion-restricted submembrane space (ΔATP); iii) at minor changes of bulk ATP, AK does not significantly contribute in setting gradients, but with further reductions in bulk ATP, flux through the AK system becomes significant in maintaining ΔATP constant (Fig. 4B). Thus, CK/AK systems, in a compartmentalized environment, can amplify and attenuate nucleotide signals at diffusion barriers [49,50].

Such modulation of nucleotide gradients at a diffusion barrier can be derived from properties of the phosphotransfer systems. The high value of the equilibrium constant for the CK reaction ($K_{CK} = 160$) implies that even a minor drop of bulk ATP under conditions of increased energy expenses would result in significant reduction of bulk CrP through the CK reaction and, thereby, a constrained diffusion flux of CrP (J_{CrP}) over intracellular diffusion barriers. This would induce a significant drop of CrP in the submembrane space, reduction of local submembrane CK activity and would result in an amplified fall of submembrane ATP ($d[ATP]_m$) compared to only small changes in bulk ATP ($d[ATP]_b$). Modulation of nucleotide signals can be expressed as a ratio between changes in submembrane over bulk ATP, i.e., $d[ATP]_m/d[ATP]_b$. In the absence of active signal modulators, a passive signal response is presented by a ratio value of 1 (Fig. 5A).

Computations with parameters of ATPase activity and diffusion coefficient performed for CK alone and cooperatively active CK and AK systems [49,84,92] revealed that the effectiveness of signal transmission driven by CK is insensitive to AK modulation at smaller variations in bulk ATP (Fig. 5A, zone of the *AK bypass*, where the signal response curves constructed for CK alone and CK + AK are superimposed). Downward deviation of the signal response curve relative to the curve constructed for CK alone indicates the contribution of the AK system into signal attenuation following a further drop in bulk ATP (Fig. 5A, zone of signal *attenuation by AK*). Such profile of interplay between phosphotransfer systems quantitatively depends on diffusion restriction values (Fig. 5B), and is in agreement with experimental observations that reduction of CK flux is accompanied by up-regulation of AK phosphotransfer [108,111].

Signal transmission over diffusion barriers is not instantaneous; it depends on diffusion coefficient rates and phosphotransfer reaction properties catalyzing signal conversion. A diffusion barrier may determine not only amplification and attenuation (tuning) of

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nucleotide signals, but also “filtering” of sustained trends in cell energetics, protecting submembrane reactions from non-sustained nucleotide fluctuations and securing an uncluttered nucleotide sensor response [112,113]. The time course of nucleotide and creatine kinase substrate levels in any point between bulk and sarcolemma can be resolved using Fick's 2nd law in combination with phosphotransfer reaction fluxes and total adenine nucleotide [ANP] and creatine derivatives [CrT] pools:

$$
\begin{aligned}
&D\frac{\partial^2[AMP]}{\partial x^2}=\frac{\partial[AMP]}{\partial t}+J_{AK}\\
&D_{Cr}=\frac{\partial^2[CrP]}{\partial x^2}=\frac{\partial[CrP]}{\partial t}-J_{CK}\\
&D\frac{\partial^2[ATP]}{\partial x^2}=\frac{\partial[ATP]}{\partial t}+J_{ATPase}+J_{CK}+J_{AK}\\
&[ATP]+[ADP]+[AMP]=[ANP]\\
&[Cr]+[CrP]=[CrT]
\end{aligned}
$$

Resolving this system of equations, based on the equilibrium for ATPase, CK and AK reactions, using apparent diffusion coefficients for ATP: $D = 1.6 \times 10^{-11}$ cm^2/s and creatine: $D_{Cr} = 2.4 \times 10^{-11}$ cm^2/s along with parameters defined above, revealed a significant delay of cytosolic signal transmission into the subsarcolemmal compartment making evident that short-living nucleotide changes that occurred in the cytosol will be essentially cutoff or low-pass filtered at a strong diffusion barrier (Fig. 5C). Brief changes in ATP levels during the cardiac contractile cycle do not communicate into changes of membrane excitability (Fig. 5D) [114]. Only changes in cell energetics approaching ~1 min in duration would be communicated through diffusion barriers into the submembrane compartment. Low-pass nucleotide signal filtering at the diffusion barrier provides a paradigm for conversion of sustained trends but not momentary fluctuations in cell energetics, a fundament of cell compartmentation defining cardiac tissue physiology.

5. Concluding remarks

In cardiac myocytes, co-localization of metabolic transducers and downstream effectors is achieved through specialized anchoring components, allowing vectorial matching of environmental signals. Sole co-localization of signal transducers and effectors may not by itself secure rapid and efficient targeting as demonstrated for voltage-dependent Na^+ channels and the Na^+–Ca^{2+} exchanger, or for adenylate cyclase and the cAMP-gated channel. To this end, an additional cellular factor, namely diffusional restriction in the proximity of macromolecular complexes, needs to be considered to comprehensively assess intracellular signaling. While the morphological basis for diffusion restrictions remains intriguing, the concept of energy transfer over diffusion barriers emerges as critical in understanding mechanisms of metabolic balance maintenance. In accord with the “divide and conquer” concept, cellular diffusion restrictions force phosphotransfer systems to play multiple roles in energy communication to compartmentalized membrane metabolic units. Under normal metabolic state they facilitate energy delivery to sarcolemmal energy consuming systems that are secluded from the cytosolic bulk space. Notably, AK is in reserve until a substantial drop in local ATP and concomitant increase in ADP recruit the AK system for local ATP support and initiation of AMP-dependent signaling cascades [86,109,110]. K_{ATP} channels anchored within sarcolemmal metabolic units spare local energy resources during excitation–contraction coupling [50,61,82]. Under stress, in response to CK and AK signals indicating that bulk cellular energetics experience a sustained energy blackout, but not in response to transient fluctuations of bulk nucleotide levels, K_{ATP} channel-driven shortening of action potentials would limit cellular energy demand [55,58,63,64,104]. Thus, spatial integration of macromolecular sarcolemmal or sarcomeric complexes, operating in diffusion-restricted intracellular compartments, with

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metabolic circuits responsible for transmission, sensing and processing of energy signals into cellular responses, ensures cardiac energy homeostasis.

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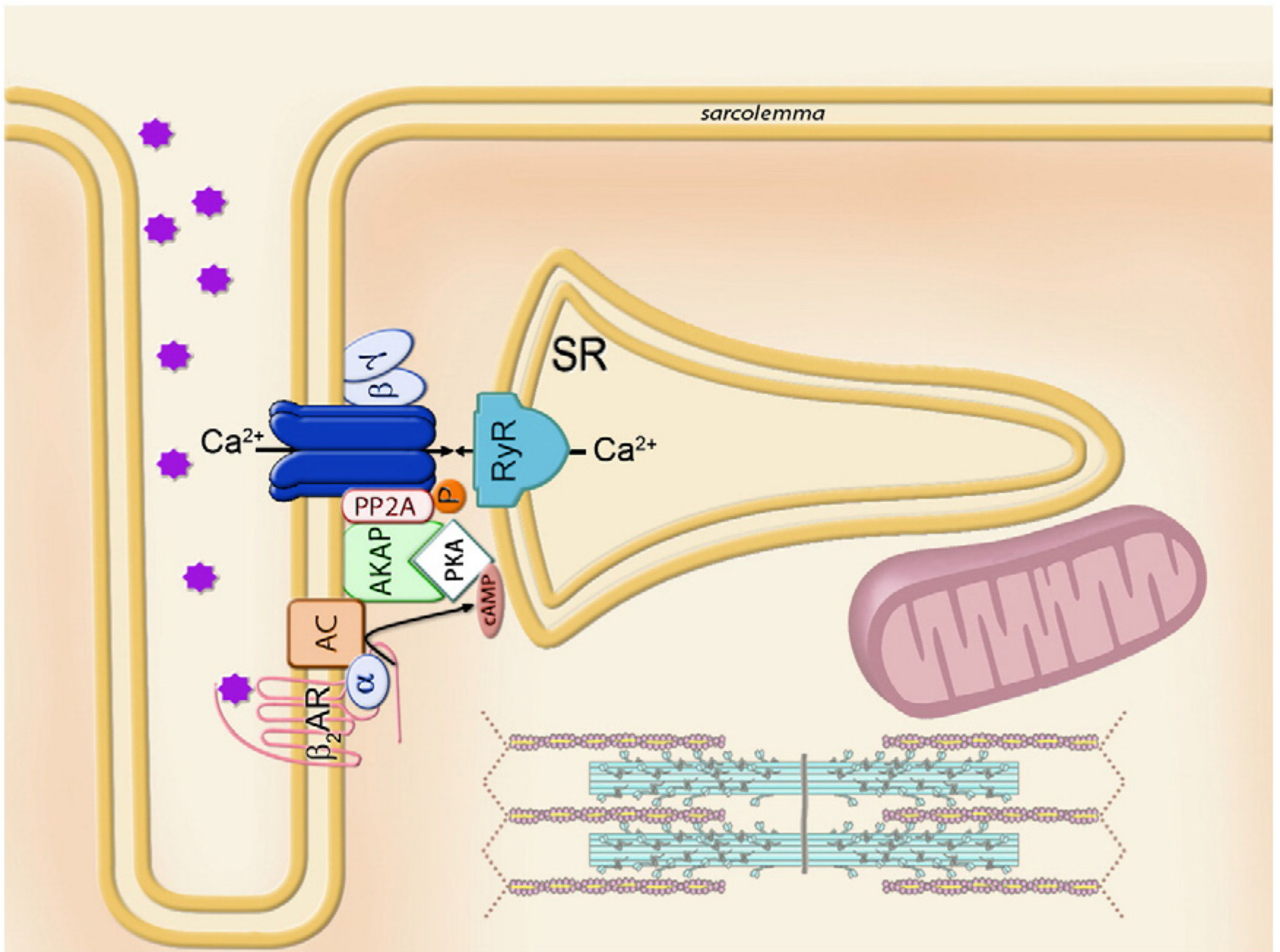

Fig. 1.

Model of a G-protein coupled signaling multiprotein module, which integrates β_2-adrenoreceptor (β_2AR) with the heteromeric G-protein (α and βγ subunits) and adenylate cyclase (AC), which by producing cAMP activates protein kinase A (PKA), anchored to the membrane through AKAP. Phosphorylation status and therefore operation of the final effector, L-type Ca^{2+} channel, is regulated by the ratio between local PKA and phosphatase (PP2A) activities. The Ca-channel macromolecular complex ensures rapid and vectorial activation of the specific effector and downstream pathways, exemplified here as Ca^{2+} release from sarcoplasmic reticulum (SR) through the ryanodine receptors (RyR).

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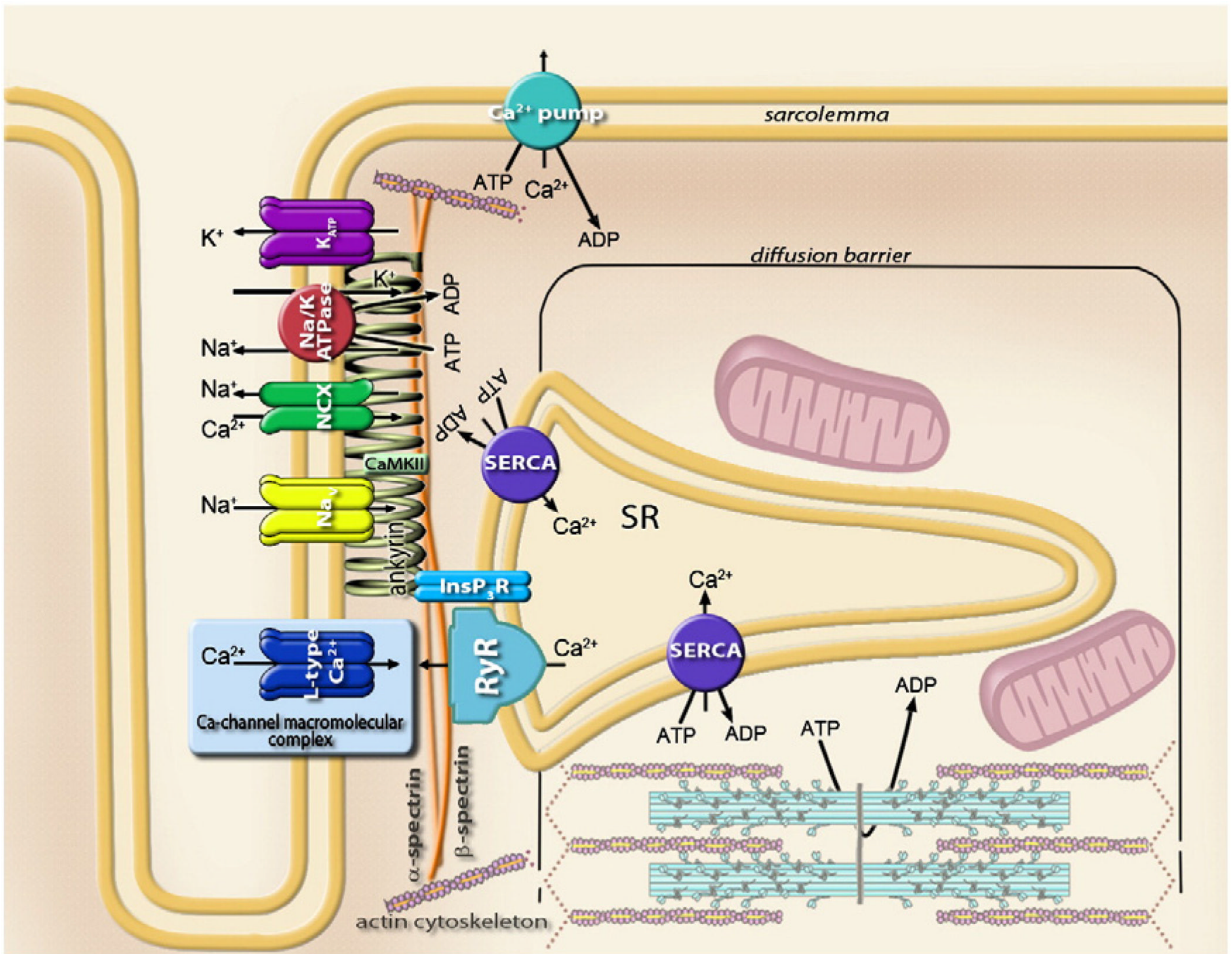

Fig. 2.

Sarcolemmal metabolic unit comprised of the cardiac ankyrin-B membrane-associated protein complex, the Ca-channel macromolecular complex (see Fig. 1) and components of the sarcoplasmic reticulum. Ankyrin bound to β_2-spectrin targets Na^+/K^+ ATPase, voltage-dependent Na^+ (Na_v) and ATP-sensitive K^+ (K_{ATP}) channels, Na^+–Ca^{2+} exchanger (NCX), IP_3 receptor (IP_3R), and, through interactions with obscurin (not shown), protein phosphatase 2A (PP2A). Coordination of calmodulin-dependent kinase (CaMKII) by β-spectrin in the proximity to Na^+ channel is also indicated.

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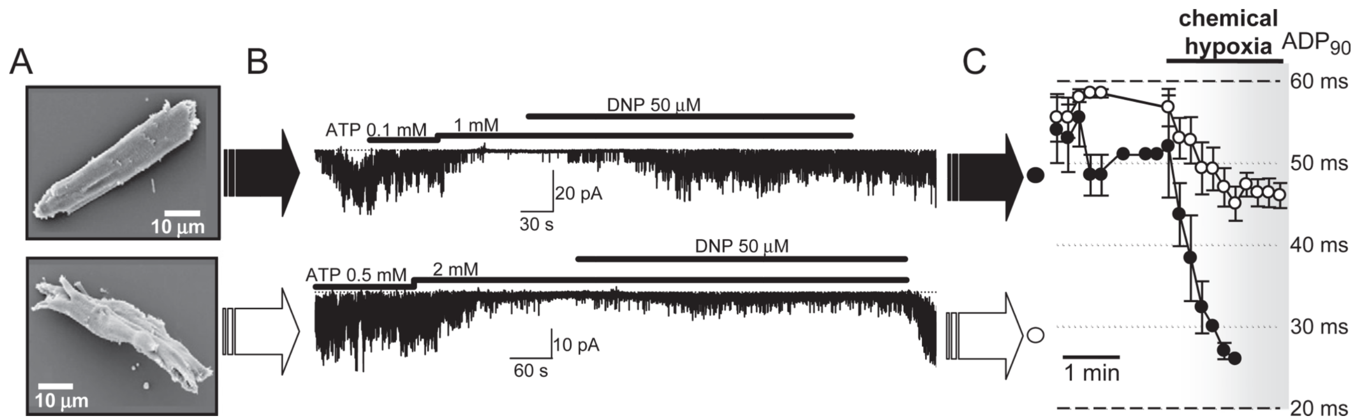

Fig. 3.

A: Remodeled ventricular myocyte from the heart of a TNFα-TG transgenic mouse manifests distorted cellular architecture (*bottom*) compared to the rod-shaped cell from control wildtype counterparts (*top*), visualized by scanning electron microscopy. B: In the open cell-attached mode of the patch-clamp technique, obtained by cell permeabilization with digitonin (Abraham et al. [55]), uncoupling of bulk mitochondria with 2,4-dinitrophenol (DNP), in the constant presence of inhibitory concentration of ATP, activated sarcolemmal K_{ATP} channel activity in cardiac myocytes from control hearts (*top*). Impeded by cellular remodeling, the communication between the cytosol and the sarcolemma resulted in a blunted K_{ATP} channel response to DNP-induced metabolic inhibition (*bottom*), despite intact intrinsic K_{ATP} channel gating properties (data not shown). C: Under chemical hypoxia, induced by DNP, APD_{90} (action potential duration measured at 90% of its amplitude) was markedly shortened in controlled (closed circlers), but not in TNFα-remodeled hearts (open circles) due to disturbed energy signals communication to sarcolemmal K_{ATP} channels (see Hodgson et al. [75] for further information).

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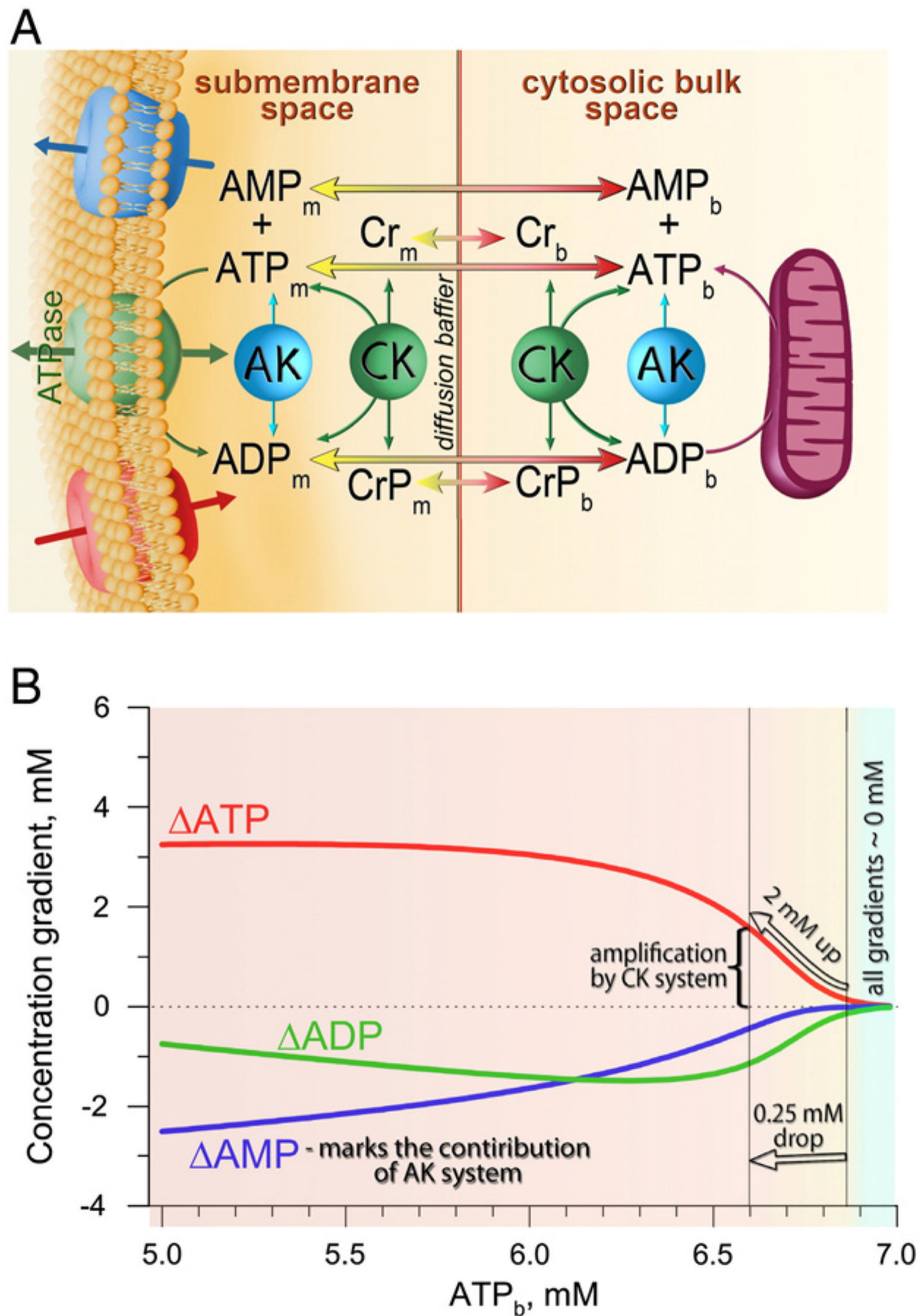

Fig. 4.

A: Diffusion of adenine nucleotides between intracellular compartments facilitated by creatine (CK) and adenylate (AK) kinase systems, implying that delivery of ATP from one cellular compartment to another, in addition to passive diffusion flux, is accompanied by influx into submembrane space of high-energy phosphate equivalents (creatine phosphate, CrP) or efflux of AMP, which then are locally involved in CK- or AK-catalyzed ATP synthesis. Indexes ‘m’ and ‘b’ denote the metabolite concentrations in the submembrane and cytosolic bulk spaces, respectively. Influx and efflux of ions through sarcolemmal channels and concomitant ATPase flux are also indicated. B: Differences in ATP, ADP and AMP concentrations (ΔATP, ΔADP and ΔAMP) between the bulk and submembrane space at

different bulk ATP concentrations (ATP_b). Positive gradient values correspond to the drop of nucleotide concentrations directed from the diffusion barrier towards the sarcolemma. Nucleotide gradients were constructed by resolving equation 6 from Selivanov et al. [49], at $J_{ATPase} = 4.7 \times 10^{-6}$ μmol/cm^2/s, $D = 1.6 \times 10^{-11}$ cm^2/s, 7 mM total nucleotide pool, 200 nm thickness of subsarcolemmal space and variable bulk ATP.

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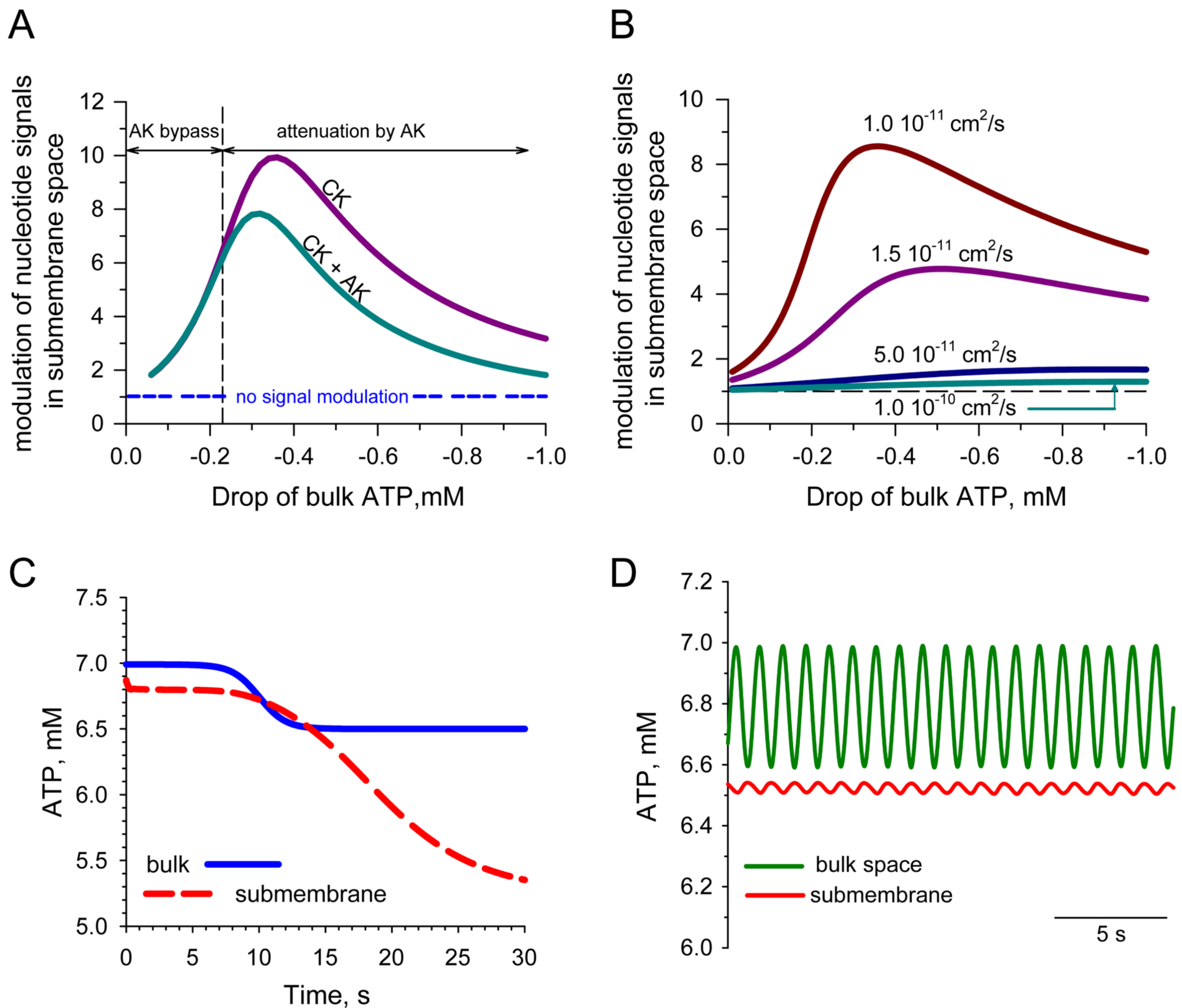

Fig. 5.

A: Effectiveness of modulation of nucleotide signals, i.e. changes of ATP in submembrane space ($\Delta[ATP]_m$) relative to a drop of ATP in bulk cytosolic levels ($\Delta[ATP]_b$), was calculated as $d[ATP]_m/d[ATP]_b$ for creatine kinase (CK) alone and co-active CK and AK systems. The horizontal dotted line corresponds to a passive signal response (i.e. no signal modulation) in the absence of systems catalyzing phosphotransfer reactions. Note that higher changes in ATP_b undergo a lower amplification, an effect enhanced by AK when a drop of ATP_b exceeds the “AK bypass” threshold. B: Modulation of nucleotide signals under co-active CK and AK systems at different values of the apparent diffusion coefficient for nucleotides in submembrane space. C: Kinetic simulation of a nucleotide signal in submembrane space in response to a sustained drop of bulk ATP. Calculations were performed by resolving the system of differential equations (in the text) using $J_{ATPase} = 4.7 \times 10^{-6}$ μmol/cm^2/s, $D = 1.6 - 10^{-11}$ cm^2/s, 200 nm thickness of subsarcolemmal space, 7 mM total nucleotide ([ANP]) and 40 mM Cr/CrP ([CrT]) pools. The nucleotide signal, generated in cytosol as 0.5 mM drop of ATP (blue solid line) is amplified in the membrane vicinity and reaches steady-state within >30 s delay (red dotted line). D: Same approach and

values of parameters were used to simulate cytosolic ATP oscillations during contraction at 1 Hz frequency that were effectively filtered out in the vicinity of the sarcolemma. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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