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Role of the cytoskeleton in communication between L-type Ca^{2+} channels and mitochondria

Helena M. Viola and Livia C. Hool

Cardiovascular Electrophysiology Laboratory, School of Anatomy, Physiology and Human Biology, The University of Western Australia, WA 6009, Australia

Summary

1. The L-type Ca^{2+} channel is the main route for Ca^{2+} entry into cardiac myocytes, which is essential for the maintenance of cardiac excitation and contraction. Alterations in L-type Ca^{2+} channel activity and Ca^{2+} homeostasis have been implicated in the development of cardiomyopathies.

2. Cardiac excitation and contraction is fuelled by adenosine-5′-triphosphate (ATP), synthesized predominantly by the mitochondria *via* the Ca^{2+}-dependent process oxidative phosphorylation. Mitochondrial reactive oxygen species (ROS) are by-products of oxidative phosphorylation and are associated with the development of cardiac pathology.

3. The cytoskeleton plays a role in communication of signals from the plasma membrane to intracellular organelles. There is good evidence that both L-type Ca^{2+} channel activity and mitochondrial function can be modulated by alterations in the cytoskeletal network.

4. Activation of the L-type Ca^{2+} channel can regulate mitochondrial function through cytoskeletal proteins as a result of transmission of movement from the β_2 subunit of the channel that occurs during activation and inactivation of the channel. An association between cytoskeletal proteins and the mitochondrial voltage dependent anion channel (VDAC) may play a role in this response.

5. The L-type Ca^{2+} channel is the initiator of contraction in cardiac muscle and VDAC is responsible for regulating mitochondrial ATP/adenosine diphosphate (ADP) trafficking. This article presents evidence that a functional coupling between the L-type Ca^{2+} channels and mitochondria may assist in meeting myocardial energy demand on a beat to beat basis.

Introduction

The cytoskeletal network is well known for modulating cell morphology, motility, intracytoplasmic transport and mitosis. It has also been proposed that cytoskeletal proteins assist with the communication of signals from the plasma membrane to intracellular organelles. Cardiac muscle has a high demand for energy. Mitochondria are complex organelles responsible for maintaining production of ATP to fuel cardiac excitation and contraction. This includes the rapid uptake of Ca^{2+} during the cardiac cycle.

The L-type Ca^{2+} channel is central to myocardial physiology. Ca^{2+} influx through the L-type Ca^{2+} channel is essential for the maintenance of cardiac excitation and contraction and mitochondrial ATP production. There is increasing evidence suggesting a role for the cytoskeleton in modulating both L-type Ca^{2+} channel activity and mitochondrial function. This article presents evidence that in addition to mediating Ca^{2+} influx, activation of the L-type Ca^{2+} channel may also modulate mitochondrial VDAC as a result of transmission of movement from the β_2 subunit of the channel to the mitochondria through cytoskeletal proteins.

The L-type Ca^{2+} channel

Role of the L-type Ca^{2+} channel in Ca^{2+} homeostasis and contraction

Ca^{2+} is a key determinant in the control of cardiac function. Maintaining Ca^{2+} homeostasis is therefore essential to life. A number of plasma membrane and intracellular Ca^{2+} channels and transporters are involved in maintaining Ca^{2+} homeostasis during the course of cardiac excitation, contraction and relaxation.[1]

The L-type Ca^{2+} channel is the main route for Ca^{2+} entry into cardiac myocytes. Ca^{2+} influx through the L-type Ca^{2+} channel shapes the long plateau phase of the ventricular action potential and the upstroke and duration of the atrial pacemaker action potentials.[2,3] In cardiac muscle, Ca^{2+} influx during depolarization of the action potential initiates the sequence of events that result in contraction.[3] Initiation of contraction requires a rapid and significant increase in intracellular Ca^{2+} from a basal concentration of approximately 100 nmol/l to 1 μmol/l.[4,5] This is achieved by a process known as Ca^{2+}-induced Ca^{2+} release (CICR), which is initiated by Ca^{2+} influx through the L-type Ca^{2+} channel in response to depolarization of the action potential.[3] Ca^{2+} influx *via* the L-type Ca^{2+} channel triggers Ca^{2+} release from sarcoplasmic reticulum (SR) stores *via* inositol triphosphate receptors (IP_3R) and ryanodine receptors (RyR).[2,5,6] IP_3R2 expression is the main IP_3R subtype expressed in atrial and ventricular myocytes.[7-9] IP_3R2 have been shown to play a large role in CICR in atrial myocytes, with IP_3R2 expression being 6-10 fold higher in atrial versus ventricular myocytes.[9] In ventricular myocytes, Ca^{2+} release from IP_3R has been demonstrated to modulate Ca^{2+} dependent transcription factors however the rate and extent of Ca^{2+} release from IP_3R alone is insufficient to result in Ca^{2+} induced Ca^{2+} release.[3,10] IP_3R and RyR are activated at submicromolar and micromolar concentrations of Ca^{2+} respectively.[6] It has been proposed

that this may assist CICR by enabling local Ca^{2+} release of one receptor to activate, and therefore amplify, Ca^{2+} release of a nearby receptor.[11] Overall, this CICR mechanism, initiated by Ca^{2+} influx through the L-type Ca^{2+} channel in response to depolarization of the action potential, facilitates rapid and significant increases in intracellular Ca^{2+} which are essential to contraction.

Cardiac muscle fibres consist of many overlapping strands of contractile proteins, including thick filaments comprised of myosin and thin filaments comprising actin and tropomyosin. Contraction occurs as a result of a complex interaction between these contractile proteins in response to SR Ca^{2+} release.[1] Ca^{2+} released from the SR binds to troponin C present on thin filaments and causes allosteric modulation of thin filament tropomyosin to unblock thick filament myosin binding sites. Myosin, powered by hydrolysing ATP, then moves along these binding sites resulting in muscle contraction. Contraction is closely followed by relaxation of the muscle fibres, which is achieved *via* removal of cytosolic Ca^{2+}. This occurs predominantly by Ca^{2+} uptake by the SR *via* the Ca^{2+}-ATPase Ca^{2+} pump.[3] Remaining Ca^{2+} is extruded *via* the Na^{+}/Ca^{2+} exchanger (NCX).[2,5]

Structure of the L-type Ca^{2+} channel

Cardiac L-type Ca^{2+} channels are heterotetrameric polypeptide complexes consisting of α_{1C}, $\alpha_2\delta$ and β_2 subunits. α_1 subunit proteins are classified into 4 classes. These include $Ca_v1.1$ (α_{1S}), $Ca_v1.2$ (α_{1C}), $Ca_v1.3$ (α_{1D}) and $Ca_v1.4$ (α_{1F}).[5,12] Cardiac muscle expresses only the dihydropyridine sensitive α_{1C} subunit which is encoded by the CACNA1C gene.[5,12] The α_{1C} subunit consists of 4 homologous motifs (1-4) each of which consist of 6 transmembrane α-helices (S1-S6) which are linked by cytoplasmic loops.[5] The 4 motifs of the α_{1C} subunit form the pore of the channel which regulates ion conductance, voltage sensing and contains binding sites for channel-modifying second messengers, toxins and drugs.[4,13,14] The $\alpha_2\delta$ subunit of the L-type Ca^{2+} channel is an accessory subunit which consists of 2 proteins, α_2 and δ which are encoded by the same gene separated by posttranslational cleavage.[15-17] The δ part is a transmembrane protein with large extracellular and short intracellular portions while α_2 is located entirely extracellular.[5,16] The 2 proteins are linked by a disulfide bond to form the $\alpha_2\delta$ subunit that associates with the α_{1C} subunit *via* surface interaction.[17] There is evidence to suggest that the α_2 subunit facilitates structural modification of the channel while δ modulates voltage dependent activation and inactivation kinetics.[17-19] The $\alpha_2\delta$ subunit plays a role in trafficking the α_{1C} subunit to the cell membrane and regulating voltage dependent activation and inactivation kinetics of the channel.[17] The β_2 subunit of cardiac L-type Ca^{2+} channels is an accessory subunit that is entirely intracellular. It is tightly bound to the cytoplasmic linker between motifs I and II of the α_{1C} subunit called the α-interacting domain (AID).[20] The β_2 subunit plays a role in regulating trafficking of the α_{1C} subunit to the cell membrane, modifying open probability of the channel and activation and inactivation kinetics.[20,21]

The L-type Ca^{2+} channel and cardiac pathology

There is a significant amount of evidence that argues that phenotypic remodeling and the development of cardiac hypertrophy can occur as a consequence of alterations in L-type Ca^{2+} channel function.[22-27] Hypertrophic stimuli have been shown to activate a program of fetal cardiac gene transcription that encodes proteins involved in contraction, Ca^{2+} handling and metabolism.[28,29] The cellular phenotypic changes associated with cardiac hypertrophy are preceded by reactivation of postnatally dormant embryonically expressed genes while several "adult" genes are repressed.[30,31] Inhibition of the L-type Ca^{2+} channel with nicardipine has been demonstrated to prevent activation of the fetal gene program by isoproterenol in neonatal rat cardiac myocytes.[22] In similar studies, inhibition of the L-type Ca^{2+} channel with nifedipine prevented cardiac hypertrophy induced by mechanical stretch in neonatal rat cardiac myocytes.[24]

Transgenic mice overexpressing the α_{1C} subunit of the L-type Ca^{2+} channel exhibit increased intracellular Ca^{2+}, that results in the development of cardiac hypertrophy and consequently heart failure.[23] Similarly, transgenic mice overexpressing the β_2 subunit of the L-type Ca^{2+} channel have been shown to develop cardiac hypertrophy which was attenuated when mice were treated with the L-type Ca^{2+} channel blocker verapamil.[25]

The L-type Ca^{2+} channel blocker diltiazem is beneficial in reducing cardiac hypertrophy and fibrosis in a mouse model of familial hypertrophic cardiomyopathy ($\alpha MHC^{403/+}$), that expresses an Arg403Gln missense mutation in one allele of the cardiac β-myosin heavy chain.[32] This mutation has been demonstrated to be associated with disrupted SR Ca^{2+} homeostasis, characterized by excess sarcomeric Ca^{2+} due to increased Ca^{2+} sequestration by the mutant sarcomere, and Ca^{2+} depletion in the SR. The disruption to SR Ca^{2+} homeostasis preceded the development of cardiac hypertrophy in $\alpha MHC^{403/+}$ mice, while early administration of diltiazem prevented the development of cardiac hypertrophy. In this model it was proposed that diltiazem inhibition of the L-type Ca^{2+} channel attenuates Ca^{2+}-induced Ca^{2+} release, limiting Ca^{2+} sequestration by the mutant sarcomere and subsequently the development of pathology.

The role of the L-type Ca^{2+} channel in the development of hypertrophy has also been studied in humans. The expression levels of α_{1C} and β_2 subunits of the L-type Ca^{2+} channel are elevated in cardiac preparations from patients with hypertrophic obstructive cardiomyopathy.[26] In addition patients with Timothy syndrome, that results from a mutation within the α_{1C} subunit of the L-type Ca^{2+} channel exhibit significant cardiac hypertrophy.[27] These findings support the notion that the L-type Ca^{2+} channel may play a significant role in the mechanisms involved in the development of pathology.

Role of Ca^{2+} in mitochondrial function

Ca^{2+} and ATP production

Cardiac excitation and contraction is powered by ATP. ATP is synthesized predominantly within mitochondria *via* a Ca^{2+}-dependent process known as oxidative phosphorylation.[33,34] Ca^{2+} enters the mitochondria *via* the mitochondrial Ca^{2+} uniporter as a result of a strong electrochemical gradient for Ca^{2+} influx.[35,36] Ca^{2+} is extruded from the mitochondria *via* the NCX.[37-39] Uptake of Ca^{2+} by the mitochondria triggers activation of three key dehydrogenases of the tricarboxylic acid (TCA) cycle including isocitrate dehydrogenase, α-ketoglutarate dehydrogenase and pyruvate dehydrogenase.[37,40] Ca^{2+} is an absolute requirement for activation of isocitrate dehydrogenase and α-ketoglutarate dehydrogenase.[41-46] Activation of the TCA cycle enhances the production of NADH, that triggers movement of electrons down complexes I through to IV of the electron transport chain (ETC) by initially donating electrons to complex I.[37,47] Electrons are also fed into the ETC *via* complex II due to the conversion of succinate to fumarate within the TCA cycle. Complex IV is the terminal electron acceptor which acts to convert oxygen to water. During this process complexes I, III and IV pump protons from the mitochondrial matrix into the intermembrane space, resulting in increased proton motive force that consists of an electrochemical potential, also known as mitochondrial membrane potential (Ψ_m), and a proton gradient. This proton motive force results in the production of ATP from ADP at complex V.[33,37,47] While the production of ATP is a Ca^{2+}-dependent process, it is recognized that Ψ_m remains highly polarized and is not influenced by Ca^{2+} under conditions of low intracellular Ca^{2+} (0–200nM).[48]

Ca^{2+} and mitochondrial ROS

During oxidative phosphorylation some of the electrons passing down the ETC leak out and react with molecular oxygen to form ROS. The term ROS generally refers to oxygen molecules in different redox states.[49-52] The production of ROS begins with the reduction of oxygen to superoxide anion ($O_2^{-}\bullet$) that is generally unstable and rapidly dismutated to hydrogen peroxide (H_2O_2). Although ROS are commonly recognized as being detrimental to the health of organisms, it is now recognized that ROS can act as signaling molecules able to stimulate and modulate a variety of biochemical and genetic systems including the regulation of signal transduction pathways, gene expression and proliferation.[53]

Under normal physiological conditions, low levels of ROS (0.05-0.07 μmol/l intracellular) are required to maintain normal cellular function.[54] This includes regulation of biochemical and genetic systems such as signal transduction pathways, gene expression and proliferation.[53] Maintenance of physiological levels of ROS is achieved due to a fine balance between ROS formation and breakdown by antioxidants.[49,51,55] These include manganese superoxide dismutase, which converts superoxide to H_2O_2, and glutathione peroxidase and peroxiredoxins 3 and 5, which convert H_2O_2 to water and oxygen.[49,51,56-59] Under these conditions, small alterations in steady-state concentration of ROS regulate signal transduction pathways, gene expression, proliferation, and cell death by apoptosis.[49,53,60]

Uninhibited increases in ROS production, in which antioxidant defenses become inadequate, lead to oxidative stress.[49,55] Increases in ROS beyond 10 μmol/l (intracellular) are associated with mitochondrial damage and damage to key macromolecules such as DNA, proteins and lipids.[54,55,61-67] This can ultimately lead to increased apoptosis and development of heart failure. Sub-lethal increases in ROS (1-10 μmol/l intracellular) can activate a number of Ca^{2+}-dependent signaling kinases and transcription factors including NFAT, serine-threonine and tyrosine kinases, CaMK and MAPK by way of thiol modification in the absence of cell death.[54,65,68] Alterations in these signaling kinases have been associated with pathological growth and progression towards cardiac hypertrophy.[28,69-78]

Mitochondria are a major source of ROS production within cardiac myocytes.[47,55,79,80] The steady state concentration of superoxide in the mitochondrial matrix has been shown to be approximately 5 to 10-fold higher than that in the cytosolic and nuclear spaces.[81] In addition to mitochondria, a number of other sites within the cell can produce ROS including NAD(P)H oxidase, xanthine oxidase and nitric oxide synthase. In vascular tissue NAD(P)H oxidase is recognized as the predominant source of superoxide generation.[82] In cardiac myocytes mitochondria have been demonstrated to play a major role in the generation of ROS during acute changes in cellular redox state.[83,84] NADPH oxidase, xanthine oxidase and nitric oxide synthase do not appear to contribute to elevated ROS production during acute changes in cellular redox state.[83,84]

Role of the cytoskeleton in cardiac function

The cytoskeleton consists of microtubules comprised of tubulin, microfilaments comprised of actin, and intermediate filaments. The cytoskeletal network is recognized as a modulator of cell morphology, motility, intracytoplasmic transport and mitosis.[85,86] It has also been proposed that cytoskeletal proteins may assist with the communication of signals from the plasma membrane to intracellular organelles.[85,87] There is good evidence that cardiac L-type Ca^{2+} channel activity can be regulated by various components of the cytoskeleton.[88-94] Furthermore, in addition to modulating cytoplasmic and mitochondrial Ca^{2+}, evidence has recently been provided for regulation of mitochondrial function by the L-type Ca^{2+} channel *via* an association through the cytoskeleton.[95]

Cytoskeletal regulation of L-type Ca^{2+} channel activity

There is good evidence to suggest a role for the cytoskeleton in modulating cell surface membrane events such that external mechanical signals may be transduced to

internal sites *via* alterations in cytoskeletal organization.[85,87] This includes the regulation of Ca^{2+} transport.

L-type Ca^{2+} channels are anchored to filamentous actin (F-actin) networks by subsarcolemmal stabilizing proteins that also tightly regulate the function of the channel.[89,90,92-94,96-98] Microtubules have been demonstrated to regulate L-type Ca^{2+} channel activity in isolated chick ventricular myocytes.[88] When microtubules are dissociated with colchicine, L-type Ca^{2+} channel activity is reduced, while myocytes exposed to the microtubule stabilizing agent taxol demonstrate increased channel activity.[88] Microfilaments also appear to regulate cardiac L-type Ca^{2+} channel activity. Depolymerization of F-actin with cytocholasin D has been shown to cause a reduction in L-type Ca^{2+} channel current in adult guinea pig ventricular myocytes.[89] The effect is attenuated when myocytes are pre-incubated with phalloidin, an inhibitor of F-actin depolymerization. In addition, neonatal cardiac myocytes isolated from transgenic mice lacking gelsolin (an actin-severing protein) exhibit increased L-type Ca^{2+} channel currents.[90] The effect is attenuated when myocytes are treated with cytochalasin D or when dialyzed intracellularly with gelsolin.

Dystrophin is a subsarcolemmal protein that links the cytoskeleton to transmembrane proteins and the plasma membrane of cardiac myocytes.[91,94,99-101] Absence of dystrophin forms the molecular basis for Duchenne muscular dystrophy (DMD), an X-linked neuromuscular disorder.[91,102] Cardiac dysfunction, particularly hypertrophy and dilated cardiomyopathy, is frequently observed in boys with DMD.[91,103-107] The cardiomyopathy is associated with cytoskeletal protein disarray and mitochondrial dysfunction. Cardiac myocytes from dystrophin-deficient (*mdx*) mice do not demonstrate altered L-type Ca^{2+} channel density assessed using patch-clamp analysis, yet a delayed inactivation rate of the current has been recorded.[91,94,108] Since the auxiliary β_2 subunit regulates inactivation of the channel and also associates with subsarcolemmal proteins,[96-98] the function of the β_2 subunit of the L-type Ca^{2+} channel may be altered as a result of the absence of dystrophin.

It therefore appears that microtubules and microfilaments play an important role in stabilizing the cardiac L-type Ca^{2+} channel protein in the plasma membrane and may assist in conformational changes in the channel protein during activation and inactivation. It was recently demonstrated that the β_2 subunit of the channel associates with actin *via* a 700 kDa subsarcolemmal stabilizing protein known as AHNAK.[96-98] β-adrenergic regulation of L-type Ca^{2+} channel activity is regulated as a result of the physical coupling between the β_2 subunit of the channel and actin *via* the carboxy-terminal region of AHNAK.[98]

Cytoskeletal regulation of mitochondrial function

Cytoskeletal proteins can regulate the subcellular distribution of mitochondria.[85,109-113] The intermediate filament linker protein plectin, which colocalizes with desmin has been observed in very close proximity to mitochondria in skeletal and heart muscle.[112,113] Mice deficient in intermediate filament linker protein desmin display abnormal accumulation of subsarcolemmal clumps of mitochondria and reduced metabolic activity in both skeletal and cardiac muscle.[114] In addition, treatment of cultured cells with agents that depolymerize intermediate filaments have been demonstrated to result in codistribution of mitochondria with peripherally located microtubulues.[110,111] There is also evidence to suggest that regulation of mitochondrial function *via* the cytoskeleton occurs as a result of docking proteins existing on mitochondria which also bind to cytoskeletal elements.[85,110,112,115-119]

Cytoskeleton and cardiomyopathy

Alterations in cytoskeletal protein organization are associated with cardiac pathology such as hypertrophy and failure. Studies performed in patients with sporadic or familial hypertrophic cardiomyopathy have revealed that mutations in the cardiac actin gene can result in cardiac hypertrophy or failure.[120] In addition, experimental models of heart failure and studies on patients with heart failure indicate that defects in myocyte subcellular organelle function are associated with the development of the pathology.[121] These include defects in extracellular matrix, sarcolemma, SR, myofibrils, mitochondria, and nuclei function.[121] Disruption of the cytoskeleton in DMD and familial cardiomyopathies due to mutations in cytoskeletal proteins are associated with reduced mitochondrial oxygen consumption and subsequently, reduced ATP production.[114,122]

Role of the cytoskeleton in regulation of mitochondrial function by the L-type Ca^{2+} channel

We have explored the mechanisms by which cytoskeletal disruption leads to abnormal mitochondrial function and compromized cardiac function.

L-type Ca^{2+} channels are anchored to cytoskeletal networks by subsarcolemmal stabilizing proteins that also regulate the function of the channel.[89,90,92-94,96-98] There is also evidence for regulation of mitochondrial function *via* the cytoskeleton as a result of docking proteins existing on mitochondria which are capable of binding to cytoskeletal elements.[85,110,112,115-119] We have demonstrated that in addition to Ca^{2+} influx, alterations in L-type Ca^{2+} channel activity are sufficient to modulate mitochondrial function *via* an association through the cytoskeleton.[95]

We activated the channel directly with application of the dihydropyridine agonist Bay K8644 (BayK(−)), depolarization of the plasma membrane after exposure of adult guinea pig ventricular myocytes to 45mM KCl, or voltage-step of the plasma membrane using patch-clamp technique. This resulted in a significant increase in mitochondrial superoxide production, NADH and metabolic activity in a Ca^{2+}-dependent manner.[95] In addition, direct activation of the channel resulted in an increase in Ψ_m (Figure 1). The increase in Ψ_m occurred in a

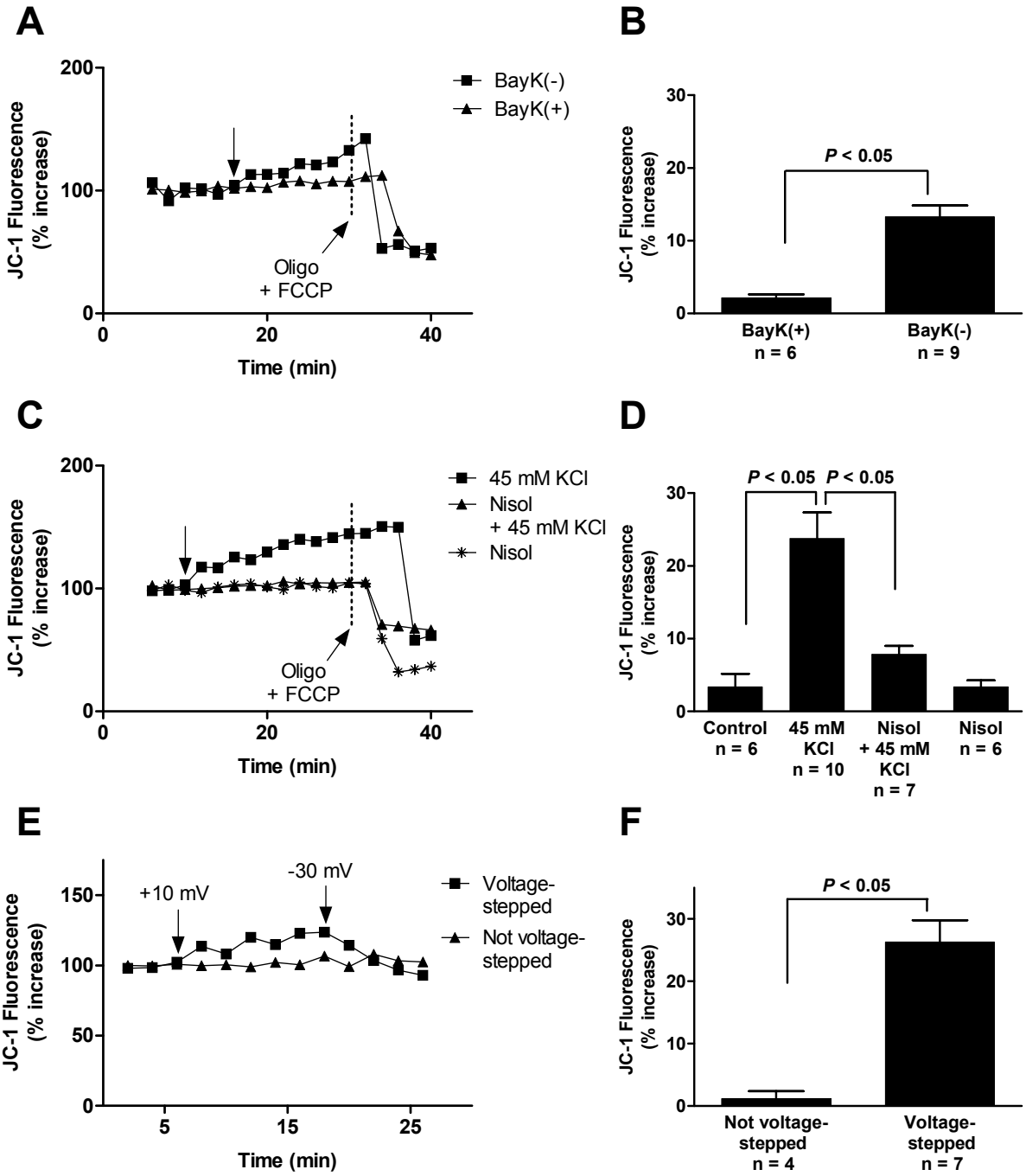

Figure 1. Direct activation of the L-type Ca^{2+} channel results in an increase in Ψ_m. ***A:*** *Ratiometric JC-1 fluorescence recorded from a myocyte before and after exposure to 2 μM BayK(−) and from another myocyte before and after exposure to 2 μM BayK(+). Arrow indicates when treatments were added. To establish that the JC-1 signal was indicative of Ψ_m 20 μM oligomycin (Oligo) and 4 μM FCCP were added at the end of each experiment to collapse Ψ_m where indicated.* ***B:*** *Mean ± SEM of changes in JC-1 fluorescence for myocytes exposed to 2 μM BayK(+) or 2 μM Bay(−) as indicated.* ***C:*** *JC-1 fluorescence recorded from a myocyte before and after exposure to 45 mM KCl, from a myocyte before and after exposure to 2 μM nisoldipine (Nisol) then 45 mM KCl, and from another myocyte before and after exposure to 2 μM Nisol. Arrow indicates when treatments were added.* ***D:*** *Mean ± SEM of changes in JC-1 fluorescence for myocytes exposed to 45 mM KCl or Nisol as indicated.* ***E:*** *JC-1 fluorescence recorded from a myocyte patch-clamped and held initially at −30 mV then voltage-stepped to +10mV followed by a voltage-step back to −30 mV as indicated with arrows (Voltage-stepped) and in another myocyte held at −30 mV and not voltage-stepped to +10 mV (Not voltage-stepped).* ***F:*** *Mean ± SEM of changes in JC-1 fluorescence for myocytes voltage-stepped and not voltage-stepped as indicated. Reproduced with permission. For further detail Viola, Arthur & Hool LC (2009).* [95]

Ca^{2+}-independent manner. Additionally, the increase in Ψ_m was attenuated in the presence of the actin filament depolymerizing agent latrunculin A or when myocytes were exposed to a synthetic peptide directed toward the AID of the L-type Ca^{2+} channel (AID-TAT), that prevents the conformational movement of the β_2 subunit of the channel during activation and inactivation of the channel.

The β_2 subunit of the L-type Ca^{2+} channel is tightly bound to the α_{1C} subunit *via* the AID.[5,20] It is also tethered to the cytoskeleton *via* subsarcolemmal stabilizing protein AHNAK.[96] The β_2 subunit plays an important role in regulating open probability of the channel and activation and inactivation kinetics.[21,123,124] In addition to Ca^{2+} influx, the L-type Ca^{2+} channel is capable of regulating mitochondrial function through cytoskeletal proteins when conformational changes in the channel occur during activation and inactivation. This appears to occur as a result of transmission of movement from the β_2 subunit of the channel through actin filaments.

We investigated the mechanisms for the alteration in Ψ_m after activation of the channel. We considered a candidate protein that could respond to movement transmitted to the mitochondria from the cytoskeleton and result in alterations in $\Psi_{m.}$ One possibility is the VDAC. VDAC, also known as mitochondrial porin, is a 32 kDa pore forming protein that resides in the outer mitochondrial membrane.[125,126] VDAC is voltage-dependent and is activated during depolarizing potentials and remains in an open state at approximately −10mV.[127,128] In the open state VDAC exhibits weak anionic selectivity and is permeant to ATP, while in the closed state VDAC exhibits weak cationic selectivity and is virtually impermeant to ATP.[127-129]

VDAC associates with the adenine nucleotide translocator (ANT) that resides in the inner mitochondrial membrane.[125] The VDAC/ANT complex is responsible for trafficking of ATP/ADP in and out of the mitochondria.[129] Cytoskeletal proteins can modify the rate of ATP production by the mitochondria because mild trypsin treatment of permeabilized rat cardiac myocytes that results in cytoskeletal disarray causes a decrease in apparent K_m for ADP.[130] There is good evidence that the cytoskeletal protein αβ-tubulin can regulate the function of VDAC because exposure of purified VDAC to tubulin causes voltage-sensitive reversible closure of VDAC assessed using the single channel patch-clamp technique.[131] Furthermore, tubulin can increase the apparent K_m for ADP in isolated mitochondria.[131] An association between VDAC and tubulin therefore appears to play a role in regulation of mitochondrial respiration.

Since regulation of Ψ_m is in part dependent on the function of VDAC,[132,133] we investigated whether VDAC plays a role in regulating mitochondrial function in response to alterations in L-type Ca^{2+} channel activity. Activation of the L-type Ca^{2+} channel with BayK(−) causes an increase in Ψ_m in isolated cardiac myocytes.[95] We examined whether directly blocking transport from the mitochondrial outer membrane could mimic the effect of BayK(−). Exposure of adult mouse cardiac myocytes to 4,4′diisothiocyano-2,2′-stilbenedisulfonic acid (DIDS) that can block VDAC caused a significant increase in Ψ_m (Figure 2) mimicking the responses evoked by exposure of cardiac myocytes to BayK(−), depolarization of the plasma membrane with application of 45 mM KCl, or voltage-step of the membrane during patch-clamp of the cell (Figure 1).[95] The L-type Ca^{2+} channel is capable of altering mitochondrial function through cytoskeletal proteins as a result of transmission of movement from the β_2 subunit of the channel that occurs during activation and inactivation of the channel.[95] An association between cytoskeletal proteins and mitochondrial protein VDAC appears to play a role in this mechanism.

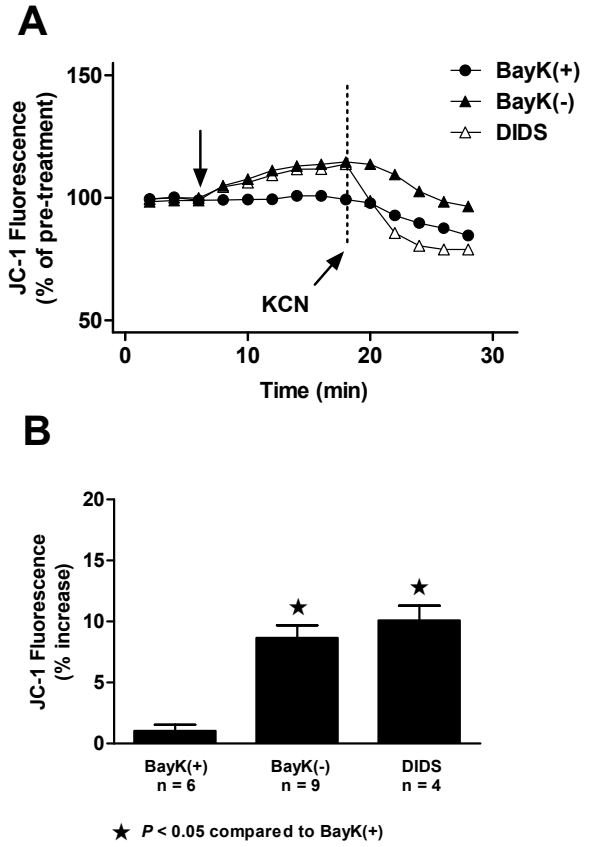

Figure 2. Exposure of myocytes to DIDS results in an increase in Ψ_m. A: *Ratiometric JC-1 fluorescence recorded from a myocyte before and after exposure to 10 μM BayK(−) or 10 μM DIDS. Vertical arrow indicates when drug was added. 4 mM KCN (K cyanide) was added to collapse Ψ_m as indicated.* ***B:*** *Mean ± SEM of changes in JC-1 fluorescence for myocytes exposed to BayK(+), BayK(−), or DIDS as indicated. DIDS: 4,4′diisothiocyano-2,2′-stilbenedisulfonic acid.*

Conclusions

The cardiac myocyte is a dynamic cell and movement during contraction influences many processes within a cell. The cytoskeleton participates by assisting in transmitting movement from the plasma membrane to intracellular organelles. Mitochondria are complex organelles

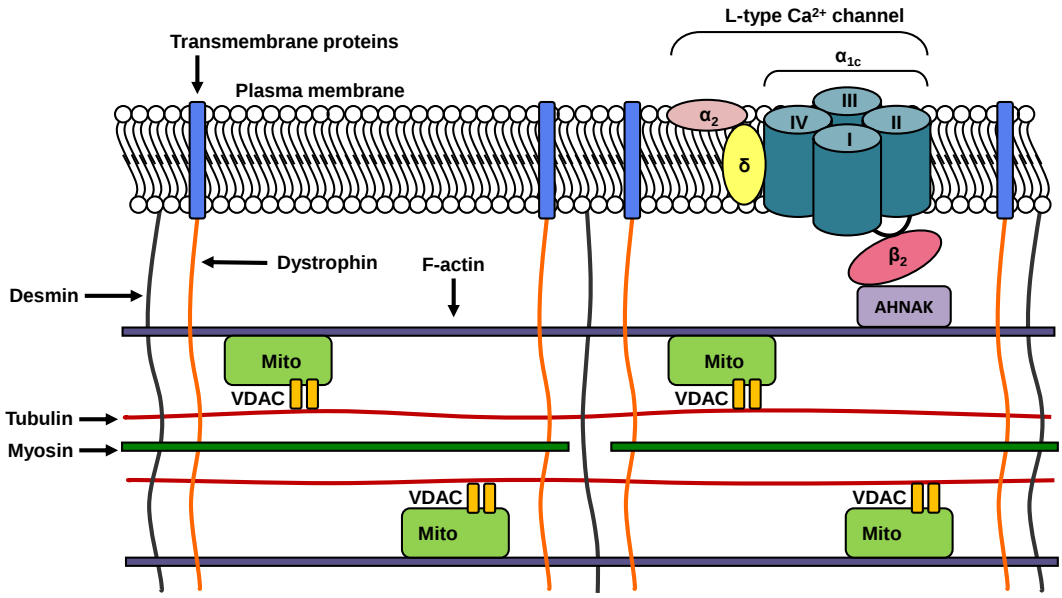

Figure 3. Proposed model explaining transmission of movement of the β_2 auxiliary subunit of the L-type Ca^{2+} channel through the cytoskeletal network to mitochondrial VDAC in response to activation of the channel. *The alpha1C (α_{1C}) subunit is shown as four transmembrane repeats I,II,III and IV. Auxiliary subunits $\alpha_2\delta$ and β_2 subunits of the channel are shown as indicated. AHNAK, 700 kDa subsarcolemmal stabilizing protein; Mito, mitochondria; VDAC, voltage-dependant anion channel (for further detail see text).*

responsible for maintaining production of ATP to meet the energy demands of the cell. This includes the rapid uptake of Ca^{2+} during the cardiac cycle. It is well recognized that the L-type Ca^{2+} channel is central to myocardial physiology and Ca^{2+} influx through the channel is a requirement for regulation of mitochondrial ATP production, mitochondrial ROS production, and contraction. Data presented here suggest that in addition to Ca^{2+} influx, activation of the L-type Ca^{2+} channel may also modulate mitochondrial function as a result of transmission of movement from the β_2 subunit of the channel to the mitochondria through cytoskeletal proteins *via* an association with VDAC (Figure 3).

Since the L-type Ca^{2+} channel is the initiator of contraction in cardiac muscle and VDAC plays a role in regulation of mitochondrial ATP/ADP trafficking, a functional coupling between the channel and the mitochondria may represent a synchronized process by which mitochondrial function is regulated to meet myocardial energy demand on a beat to beat basis.

Disruption to the sequence of communication between the L-type Ca^{2+} channel, cytoskeletal proteins, VDAC and the mitochondria, may contribute to reduced mitochondrial oxygen consumption, and subsequently reduced ATP production, that is observed in cardiac pathology associated with disrupted cytoskeletal architecture.

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Author for correspondence:

Assoc. Prof. Livia Hool
Physiology M311
School of Anatomy, Physiology and Human Biology
The University of Western Australia
35 Stirling Highway
Crawley, WA 6009,
Australia

Tel: +61 8 6488 3307
Fax: +61 8 6488 1025
E-mail: livia.hool@uwa.edu.au