How does calcium regulate mitochondrial energetics in the heart?

Running Head: Ca^{2+} and cardiac energetics

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Abstract

Maintenance of cellular calcium homeostasis is critical to regulating mitochondrial ATP production and cardiac contraction. The ion channel known as the L-type calcium channel is the main route for calcium entry into cardiac myocytes. The channel associates with cytoskeletal proteins that assist with the communication of signals from the plasma membrane to intracellular organelles, including mitochondria. This article explores the role of calcium and the cytoskeleton in regulation of mitochondrial function in response to alterations in L-type calcium channel activity. Direct activation of the L-type calcium channel results in an increase in intracellular calcium and increased mitochondrial calcium uptake. As a result, mitochondrial NADH production, oxygen consumption and reactive oxygen species production increase. In addition the L-type calcium channel is able to regulate mitochondrial membrane potential via cytoskeletal proteins when conformational changes in the channel occur during activation and inactivation. Since the L-type calcium channel is the initiator of contraction, a functional coupling between the channel and mitochondria via the cytoskeleton may represent a synchronised process by which mitochondrial function is regulated in addition to calcium influx to meet myocardial energy demand on a beat to beat basis.

Keywords

L-type calcium channel, calcium, cytoskeleton, mitochondria,

Introduction

Maintenance of calcium homeostasis is critical to regulating mitochondrial adenosine triphosphate (ATP) production and cardiac excitation and contraction. It is therefore essential to life. The L-type calcium channel is the main route for calcium entry into cardiac myocytes. Calcium influx via the channel initiates the sequence of events that result in contraction, including the production of ATP that powers cardiac excitation and contraction. ATP is synthesised by the mitochondria via a calcium-dependent process known as oxidative phosphorylation. Cardiac muscle has a high demand for energy. As such, mitochondria are capable of rapid uptake of calcium during the cardiac cycle.

The cytoskeletal network is a complex of structural proteins that modulate cell morphology. Cytoskeletal proteins also regulate cell motility, intracytoplasmic transport and mitosis. It has been proposed that cytoskeletal proteins assist with the communication of signals from the plasma membrane to intracellular organelles. The L-type calcium channel is tethered to cytoskeletal proteins that also regulate channel function. Therefore the channel plays a significant role in the regulation of intracellular calcium but can also communicate with intracellular proteins. This article explores the role of the L-type calcium channel and the cytoskeleton in regulation of mitochondrial function.

Role of calcium in cardiac function

Calcium is critical to maintaining cardiac function. Maintaining calcium homeostasis during the course of cardiac excitation, contraction and relaxation is essential to life. This involves a number of plasma membrane and intracellular calcium channels and transporters [1]. Initiation of contraction requires a rapid and significant increase in intracellular calcium from a basal concentration of approximately 100 nM to 1 μ M [2, 3]. This is achieved by a process known as calcium-induced calcium release (CICR), which is initiated by calcium influx through the L-type calcium channel in response to depolarisation of the action potential [4]. Calcium influx via the L-type calcium channel triggers calcium release from sarcoplasmic reticulum (SR) stores via inositol triphosphate receptors (IP₃R) and ryanodine receptors (RyR) [3, 5, 6]. IP₃R and RyR are activated at submicromolar and micromolar concentrations of calcium respectively [6]. This assists CICR by enabling local calcium release of one receptor that further amplifies the signal by inducing calcium release from a nearby receptor [7].

The CICR process initiates contraction. This involves a complex interaction between cardiac muscle fibre contractile proteins including thick filament comprised of myosin and thin filament comprising actin and tropomyosin [1]. Following release from the SR calcium binds to troponin C present on thin filaments and allosterically modulates tropomyosin to unblock thick filament myosin binding sites. Myosin, powered by hydrolysing adenosine-5'-triphosphate (ATP), then moves along the myosin binding sites resulting in muscle contraction. Contraction is closely followed by removal of cytosolic calcium and subsequent relaxation of the muscle fibres. Removal of cytosolic calcium occurs mainly by uptake into the SR via calcium-ATPase, with remaining calcium being extruded via the sodium/calcium exchanger (NCX) or uptake by the mitochondria via the mitochondrial calcium uniporter (MCU) [3-5].

Other channels that contribute to intracellular calcium homeostasis are the transient receptor potential (TRP) channels and T-type calcium channels. TRP channels are a group of non-selective plasma membrane cation channels activated by temperature, osmolarity, mechanical stress and noxious stimuli that conduct calcium [8]. The main subfamilies are canonical (TRPC), vanilloid (TRPV) and melastatin-related (TRPM) channels. In the heart a number of TRP channels have been identified. These include TRPM4 [9], TRPC3 and 5 [10], TRPC7 [11], and TRPM7 [12]. Activation of TRP channels depolarises the resting membrane potential and enhances calcium entry sufficient to alter intracellular calcium levels and cell signaling [13]. T-type calcium channels play an important role in intracellular calcium homeostasis during cardiac development. Expression of T-type calcium channels decreases following birth, but increases when the heart is exposed to pathological stimuli leading to the development of cardiac hypertrophy and failure [3, 14].

Calcium, mitochondria and the TCA cycle

Cardiac excitation and contraction is powered by ATP. ATP is synthesised within mitochondria via oxidative phosphorylation [15, 16]. Oxidative phosphorylation is a calcium-dependent process. Calcium uptake into the mitochondria occurs via the MCU as a result of a strong electrochemical gradient for calcium influx [17, 18]. This triggers activation of three key tricarboxylic acid (TCA) cycle enzymes including isocitrate dehydrogenase, α -ketoglutarate dehydrogenase and pyruvate dehydrogenase [19, 20]. Activation of isocitrate dehydrogenase and α -ketoglutarate dehydrogenase is dependent on calcium [21-26]. Activation of the TCA cycle results in increased production of reduced nicotinamide adenine dinucleotide (NADH) from nicotinamide adenine dinucleotide (NAD⁺), triggering movement of electrons down complexes I through to IV of the electron transport chain (ETC). Electrons

are initially donated to complex I as a result of a series of oxidation-reduction reactions [19, 27]. Electrons also enter the ETC via complex II due to the conversion of succinate to fumarate within the TCA cycle. Electrons from complex I and II are fed to complex III via coenzyme Q. Electrons from complex III are then fed via cytochrome c to complex IV, the terminal electron acceptor which acts to convert oxygen to water. Complexes I, III and IV pump protons from the mitochondrial matrix into the intermembrane space, establishing a 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 is used by complex V to convert ADP into ATP on the matrix side of the inner mitochondrial membrane [15, 19, 27]. ATP is released into the cytosol by the adenine nucleotide transporter (ANT) that resides in the inner mitochondrial membrane and the voltage-dependent anion channel (VDAC) that resides in the outer mitochondrial membrane [28-30]. ATP is generated as a result of the calcium-dependent oxidative phosphorylation process. Therefore cellular calcium homeostasis plays an important role in regulating mitochondrial ATP production. However it is recognised that Ψ_m remains highly polarized and is not influenced by calcium under conditions of low intracellular calcium (0–200 nM) [31].

Mitochondrial oxidative phosphorylation generates ATP that fuels cardiac excitation and contraction. During the oxidative phosphorylation process some of the electrons passing down the ETC react with and reduce molecular oxygen to form reactive oxygen species (ROS) [32-35]. This begins with the reduction of oxygen to superoxide anion ($O_2^{\cdot-}$) followed by rapid dismutation to comparatively more stable hydrogen peroxide (H_2O_2). The production of mitochondrial ROS is a calcium-dependent process. Exposure of rat neonatal cardiac myocytes to calcium ionophore A23187 results in elevated production of ROS [36]. Treatment of isolated rat heart mitochondria with high concentrations of calcium also results in increased production of ROS [37]. Therefore cellular calcium homeostasis plays an important role in regulating mitochondrial ROS production.

Regulation of mitochondrial energetics by the L-type calcium channel

Calcium-dependent regulation of mitochondrial energetics

Activation of the L-type calcium channel is sufficient to regulate mitochondrial function [38-40]. Mitochondria are capable of rapid uptake of calcium during the cardiac cycle. Activation of the L-type calcium channel after exposure of adult guinea pig or mouse ventricular myocytes to dihydropyridine agonist BayK(-) has been demonstrated to cause an increase in both intracellular and mitochondrial calcium [38-40]. Calcium is necessary for activation of

TCA cycle enzymes and production of NADH. In addition the ATP synthase requires calcium for the reduction of ADP to ATP at complex V (ref) Consistent with this and the effect of elevated intracellular and mitochondrial calcium, direct activation of the L-type calcium channel has been shown to result in increased NADH production, oxygen consumption and ROS production in adult guinea pig and mouse ventricular myocytes [38-40]. Therefore alterations in L-type calcium activity appear to play an important role in regulating calcium-dependent mitochondrial function.

Calcium-independent regulation of mitochondrial energetics

Mitochondrial proton motive force is used by complex V to convert ADP to ATP. This proton motive force consists of an electrochemical potential, also known as the mitochondrial membrane potential (Ψ_m), and a proton gradient. Activation of the L-type calcium channel by exposure to either BayK(-), depolarisation of the plasma membrane with high K^+ solution or voltage-stepping the plasma membrane from -30 to +10 mV using the patch clamp technique has been shown to cause an increase in Ψ_m in adult guinea pig ventricular myocytes [38]. The response could be reversed when myocytes were voltage-stepped back to -30 mV. Interestingly, increased Ψ_m in response to activation of the L-type calcium channel was attenuated when myocytes were exposed to the channel antagonist nisoldipine, but unaffected when myocytes were exposed to the mitochondrial calcium uniporter blocker Ru360 [38]. Further to this, activation of the L-type calcium channel caused an increase in Ψ_m in myocytes under calcium-free conditions [38]. These results suggested that the increase in Ψ_m associated with activation of the L-type calcium channel was not dependent upon changes in calcium transport. Activation of the L-type calcium channel caused an increase in Ψ_m under conditions where intracellular and extracellular calcium was depleted and when myocytes were also exposed to the fast inward sodium channel blocker tetrodotoxin and Na^+/H^+ exchange blocker amiloride confirming that alterations in Ψ_m were not occurring due to changes in sodium transport [38]. It is well recognised that increased mitochondrial calcium uptake is associated with increased TCA cycle activation and increased NADH production that can lead to an increase in Ψ_m . However Ψ_m can function independently of changes in mitochondrial calcium when basal calcium is approximately 0-300nM (balaban ref). These data suggest that in addition to modulating calcium influx, activation of the L-type calcium channel may also play an important role in regulating Ψ_m , independently of calcium uptake by the mitochondria.

Role of the cytoskeleton in regulation of mitochondrial energetics

The cytoskeleton consists of microtubules comprised of tubulin, microfilaments comprised of actin, and intermediate filaments. The cytoskeletal network is recognised as a modulator of cell morphology, motility, intracytoplasmic transport and mitosis [41, 42]. In mature muscle, cytoskeletal elements extend from the Z disks to the plasma membrane, traversing cellular organelles such as t-tubules, sarcoplasmic reticulum and mitochondria [43]. It has been proposed that cytoskeletal proteins assist with the communication of signals from the plasma membrane to intracellular organelles [41, 44]. L-type calcium channels are anchored to cytoskeletal networks by subsarcolemmal stabilising proteins enabling regulation of cardiac L-type calcium channel activity by various components of the cytoskeleton [45-54]. Disruption of the cytoskeletal network by depolymerisation of actin filaments or disruption of microtubules results in alterations in L-type calcium channel currents (refs). The channel associates with F-actin via a 700 kDa protein known as AHNK [50]. Cytoskeletal proteins can also regulate the subcellular distribution of mitochondria [41, 55-59]. Additionally, cytoskeletal elements are able to regulate mitochondrial function via docking proteins that bind to cytoskeletal elements [41, 56, 58, 60-64]. The absence of cytoskeletal proteins is associated with cytoskeletal disarray and poor energy supply by the mitochondria as is observed in desmin-null mice (ref) and muscular dystrophy cardiomyopathy (ref).

Since there is good evidence that cytoskeletal elements associate with and regulate L-type calcium channel and mitochondrial function, we explored a potential role of the cytoskeleton in mediating alterations in Ψ_m in response to alterations in L-type calcium channel activity. We investigated whether increased Ψ_m in response to activation of the L-type calcium channel activity was mediated by F-actin networks. Exposure of adult guinea pig cardiac myocytes to the actin depolymerising agent latrunculin A under calcium-free conditions completely attenuated the elevated Ψ_m in response to activation of the L-type calcium channel [38]. These results suggest alterations in Ψ_m in response to activation of the channel may involve the cytoskeletal element F-actin.

Conformational movement of the β_2 subunit of the L-type calcium channel as a trigger for alterations in Ψ_m

Cardiac L-type calcium channels are heterotetrameric polypeptide complexes comprising α_{1C} , $\alpha_{2\delta}$ and β_2 subunits. The α_{1C} subunit consists of 4 homologous motifs (I-IV) each of which consist of 6 transmembrane α -helices (S1-S6) which are linked by cytoplasmic loops [3]. 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 [2, 3, 5, 65, 66]. The β_2 subunit is tightly bound to the cytoplasmic linker between

motifs I and II of the α_{1C} subunit called the α -interacting domain (AID) [3, 67]. The β_2 subunit of the channel is tethered to F-actin via AHNAK [50]. The β_2 subunit plays an important role in regulating open probability of the channel and activation and inactivation kinetics [68-70].

Activation of the L-type calcium channel is sufficient to regulate Ψ_m , independently of calcium uptake by the mitochondria [38]. Since there is good evidence that cytoskeletal elements physically associate with both the L-type calcium channel and mitochondria, we examined whether increases in Ψ_m in response to activation of the L-type calcium channel occur as a result of conformational movement of the β_2 subunit of the channel that occurs during activation and inactivation.

We synthesized a peptide directed against the AID of the L-type calcium channel (AID-TAT) where the α_{1C} and β_2 subunits of the channel associate [71]. Exposure of adult guinea pig cardiac myocytes to AID-TAT for 30 min to block the association between the α_{1C} and β_2 subunits completely attenuated the increase in Ψ_m in response to activation of the L-type calcium channel [38]. These results suggest that alterations in Ψ_m in response to activation of the L-type calcium channel are dependent on a physical coupling between the L-type calcium channel and F-actin filaments. The β_2 subunit plays an important role in regulating activation and inactivation kinetics. The β_2 subunit undergoes conformational movement during the course of activation and inactivation. Confirmation that the peptide was binding the AID region was evident with the finding that cardiac myocytes exposed to the AID-TAT peptide exhibited a delayed inactivation rate of the L-type calcium channel current [38]. These data suggest alterations in Ψ_m in response to activation of the L-type calcium channel occur through conformational movement of the β_2 subunit of the channel.

We therefore propose that in addition to calcium influx, the L-type calcium channel is able to regulate Ψ_m 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 to the mitochondria.

The role of mitochondrial VDAC in regulating Ψ_m in response to alterations in L-type calcium channel activity

Activation of the L-type calcium channel is sufficient to regulate Ψ_m , independently of calcium uptake by the mitochondria [38]. This response appears to be mediated by transmission of conformational movement of the β_2 subunit of the channel that is tethered to the cytoskeleton to the mitochondria, via the cytoskeletal network. Since there is also good evidence that

cytoskeletal elements physically associate with the mitochondria, we investigated whether the outer mitochondrial membrane protein VDAC could respond to movement transmitted from the L-type calcium channel via the cytoskeleton and result in alterations in Ψ_m .

Mitochondrial VDAC is a 32 kDa pore forming protein that resides in the outer mitochondrial membrane [28, 29]. VDAC is activated during depolarising potentials and remains in an open state at approximately -10 mV [72, 73]. VDAC is permeant to ATP in the open state due to weak anionic selectivity, and is virtually impermeant to ATP in the closed state due to weak cationic selectivity [30, 72, 73]. VDAC associates with the inner mitochondrial membrane protein ANT [28]. The VDAC/ANT complex is responsible for shuttling of ATP/ADP in and out of the mitochondria [30]. There is good evidence that cytoskeletal proteins can modify the rate of mitochondrial ATP production. Exposure of permeabilised rat cardiac myocytes to trypsin to induce cytoskeletal disarray causes a decrease in apparent K_m for ADP [74], while tubulin can increase the apparent K_m for ADP in isolated mitochondria [75]. There is also good evidence that cytoskeletal proteins can regulate the function of VDAC. Exposure of purified VDAC to tubulin causes voltage-sensitive reversible closure of VDAC assessed using the single channel patch-clamp technique [75]. An association between VDAC and cytoskeletal proteins therefore appears to play a role in regulation of mitochondrial ATP production.

There is also good evidence that alterations in Ψ_m may be regulated by VDAC. Exposure of rat cardiac isolated mitochondria to GSK-3 inhibitors to dephosphorylate VDAC has been demonstrated to decrease VDAC transport of ATP across the outer mitochondrial membrane resulting in a decrease in ATP consumption and subsequently a decrease in Ψ_m [76, 77].

We investigated whether VDAC plays a role in regulating Ψ_m in response to alterations in L-type calcium channel activity. Activation of the channel by exposure of ventricular myocytes to BayK(-) has been demonstrated to cause an increase in Ψ_m [38]. We investigated whether directly blocking anion transport from the outer mitochondrial membrane via VDAC could mimic the effect of BayK(-). Exposure of adult mouse ventricular myocytes to VDAC blocking agent 4,4'-diisothiocyano-2,2'-stilbenedisulfonic acid (DIDS) caused a significant increase in Ψ_m [78]. The response mimicked that observed in response to direct activation of the L-type calcium channel [38]. These data suggest VDAC may play a role in regulating alterations in Ψ_m in response to activation of the L-type calcium channel.

In addition to calcium influx, the L-type calcium channel is able to regulate Ψ_m through cytoskeletal proteins when conformational changes in the channel occur during activation

and inactivation as a result of transmission of movement from the β_2 subunit of the channel to the mitochondria. We propose that mitochondrial VDAC may play a role in this mechanism by responding to movement transmitted from the L-type calcium channel via the cytoskeleton resulting in alterations in Ψ_m .

Conclusion

Calcium influx via the L-type calcium channel initiates excitation and contraction in the heart. ATP synthesised by the mitochondria via a calcium-dependent process known as oxidative phosphorylation fuels contraction. Cardiac muscle has a high demand for energy. As such, mitochondria are capable of rapid uptake of calcium during the cardiac cycle.

Direct activation of the L-type calcium channel can regulate mitochondrial function. Activation of the channel causes an increase in intracellular calcium, mitochondrial calcium, NADH production, oxygen consumption and ROS production in ventricular myocytes [38-40]. Therefore alterations in L-type calcium channel activity appear to play an important role in regulating calcium-dependent mitochondrial function.

The cytoskeletal network is well known for modulating cell morphology, cell motility, intracytoplasmic transport and mitosis. We have provided evidence for a role for the cytoskeleton in regulating Ψ_m in response to activation of the L-type calcium channel, which does not appear to require calcium. We propose that in addition to calcium influx, the L-type calcium channel is able to regulate Ψ_m via 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 to the mitochondria. Mitochondrial VDAC may play a role in this mechanism by responding to movement transmitted from the L-type calcium channel via the cytoskeleton resulting in alterations in Ψ_m .

Since the L-type calcium channel is the initiator of contraction and mitochondrial VDAC plays a role in regulation of mitochondrial ATP/ADP shuttling, a functional coupling between the channel and the mitochondria via the cytoskeleton may represent a synchronised process by which mitochondrial function is regulated in addition to calcium influx in order to meet myocardial energy demand on a beat to beat basis.

Acknowledgments

This study was supported by grants from the National Health and Medical Research Council of Australia and Australian Research Council. Livia Hool is an Australian Research Council Future Fellow and Honorary National Health and Medical Research Council Senior Research Fellow. Helena Viola is recipient of a National Heart Foundation of Australia Postdoctoral Fellowship.

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Figures

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Figure Legends

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