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Abstract

Cardiovascular disease is the leading cause of death in the Western world. The incidence of cardiovascular disease is predicted to further rise with the increase in obesity and diabetes and with the ageing population. Even though the survival rate from ischemic heart disease has improved over the past 30 years, many patients progress to a chronic pathological condition, known as cardiac hypertrophy that is associated with an increase in morbidity and mortality. Reactive oxygen species (ROS) and calcium play an essential role in mediating cardiac hypertrophy. The L-type calcium channel is the main route for calcium influx into cardiac myocytes. There is now good evidence for a direct role for the L-type calcium channel in the development of cardiac hypertrophy. Cysteines on the channel are targets for redox modification and glutathionylation of the channel can modulate the function of the channel protein leading to the onset of pathology. The cysteine responsible for modification of L-type calcium channel function has now been identified. Detailed understanding of the role of cysteines as possible targets during oxidative stress may assist in designing therapy to prevent the development of hypertrophy and heart failure.

Key words

Ca\textsubscript{1.2} protein, cysteine, glutathionylation, heart, hypertrophy, L-type calcium channel, oxidative stress, structure-function relationship

Introduction

The term cardiovascular disease refers to the disease of the heart and blood vessels. Cardiovascular disease is the leading cause of death in the Western world. Approximately 17.3 million people died from cardiovascular disease in 2013, accounting for 30 per cent of all deaths globally, of which 7.3
million people died due to ischemic heart disease (1). Ischemic heart disease is the leading cause of mortality and morbidity. It occurs due to the inadequate supply of myocardial oxygen and blood (2). Survival rates from ischemic heart disease have improved over the past decades, but many survivors progress to a chronic pathological condition, known as cardiac hypertrophy that is an independent risk for increased mortality according to the Framingham Heart Study (3-5). Previous studies have shown that reactive oxygen species (ROS) and calcium play a significant role in mediating the onset of cardiac hypertrophy (6). ROS are recognised as activators of various signalling pathways associated with the development of pathophysiologic cardiac conditions (7). ROS can alter numerous cellular functions by altering the redox state of key proteins (8), such as ion channels.

Oxidative stress is associated with an increase in intracellular Ca$^{2+}$, a second messenger, that activates transcription factors, resulting in increased protein synthesis, thereby leading to cardiac myocyte remodelling and hypertrophy (9-13). The L-type Ca$^{2+}$ channel is the main route for Ca$^{2+}$-influx into the cardiac myocytes, and it has been proposed to play a significant role in the development of cardiac hypertrophy. This review discusses the contribution of ROS and Ca$^{2+}$ in cardiac physiology and in the development of pathological conditions of the heart with particular reference to the role of cysteines on the cardiac L-type Ca$^{2+}$ channel in mediating oxidative stress responses.

**Role of reactive oxygen species in cardiac function**

Reactive oxygen species such as superoxide anion ($O_2^{-}$) and hydroxyl radical (‘OH) are extremely reactive molecules because of their unpaired electrons. Superoxide is the primary form of ROS, formed by the one electron reduction of molecular oxygen. Mitochondria are a significant source of superoxide production in mammalian cardiac myocytes (7, 14), because ROS are produced as a by-product of mitochondrial oxidative phosphorylation (15). ROS can also be produced outside the mitochondria from a number of cytosolic enzymes including xanthine oxidase and NAD(P)H oxidase and cytochrome P450-dependent oxygenases. $O_2^{-}$ can be formed, when a single electron is directly
transferred to oxygen by reduced coenzymes or in prosthetic groups (for example, flavins or iron sulphur clusters). Peroxides and hypochlorous acid (HOCl) are reduced forms of superoxide (16). Hydrogen peroxide (H$_2$O$_2$) and peroxynitrite (ONOO$^-/ONOOH$) are more stable, nonradical forms of ROS, they lack the free unpaired electron and thus possess oxidizing properties. H$_2$O$_2$ is produced from O$_2$•$^-$ by dismutation, which can occur spontaneously at low pH or can be catalysed by manganese superoxide dismutase (MnSOD) in mitochondria and in the cytosol. H$_2$O$_2$ can be partially reduced to hydroxyl radicals (•OH) and HOCl, through Fenton or Haber-Weiss reactions, in the presence of metal ions, or completely reduced to water. Superoxide can also react with nitric oxide (•NO) to form peroxynitrite (ONOO$^-/ONOOH$) (17, 18). Reactive oxygen and nitrogen species are key elements of many cell signalling pathways.

Under physiological conditions, ROS concentrations are tightly controlled by antioxidants. The low intracellular concentration of ROS in the range of 0.001-0.5 µM enables their role as second messengers in signal transduction and physiological cell signalling (19, 20). Oxidative stress is defined as an imbalance between ROS production and the cell’s antioxidant defence, leading to an altered redox status (16, 21). It is caused by diminished levels of antioxidants and co-factors or by increased production of ROS (22).

A slight increase in the intracellular concentration of ROS (1-10µM) causes mild oxidative stress (a sub-lethal concentration) where the cell can adapt to the changed environment. A concentration of ROS exceeding 10-100µM damages molecular targets, such as lipids, proteins and carbohydrates, leading to impaired cell function and apoptosis or necrosis (19, 23). ROS activates numerous cell-signalling pathways that trigger transcription factors and enhance the expression of specific genes responsible for growth, proliferation and apoptosis of cells (15, 24), oxidising cysteine rich motifs on many proteins, such as growth factors and cytokine receptors, further activating signalling pathways (25, 26).
**Ca\textsuperscript{2+} as a second messenger**

Calcium ions play an essential role in many cellular processes. Calcium is vital to excitation, contraction and relaxation of the cardiac muscle. Since cardiac sarcoplasmic reticulum is less extensive and less precisely arranged in relation to the cross-banded pattern of the myofibrils than it is in skeletal muscle, cardiac muscle is reliant on Ca\textsuperscript{2+} influx for contraction. Depolarisation of the membrane of cardiomyocytes stimulates opening of voltage-gated Ca\textsuperscript{2+} channels (27). The L-type Ca\textsuperscript{2+} channel is the primary route for Ca\textsuperscript{2+} influx into the cardiac myocytes (28). Influx of calcium ions through the L-type Ca\textsuperscript{2+} channel triggers Ca\textsuperscript{2+} release from the sarcoplasmic reticulum (SR) stores through the inositol 1,4,5-triphosphate or ryanodine receptor 2 (RyR2). The amplifying process is termed Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release. An increase in intracellular concentration of calcium ions shapes the long plateau phase of the ventricular action potential, along with the upstroke of the atrial pacemaker action potential, regulating the electrical signal that determines the cardiac rhythm (10, 29, 30).

The cardiac muscle consists of orderly arranged contractile proteins composed of thick myosin filaments and overlapping thin actin and tropomyosin filaments. Increased Ca\textsuperscript{2+} concentration facilitates the binding of Ca\textsuperscript{2+} to troponin C, thereby inducing the contraction of cardiac muscle (31, 32). The removal of cytosolic calcium facilitates the relaxation of cardiac muscles and precisely controls the intracellular Ca\textsuperscript{2+} concentration crucial for many signal transduction pathways and cellular activities. This occurs by the uptake of calcium into the sarcoplasmic reticulum via the Ca\textsuperscript{2+}-ATPase, extrusion from the cell through the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger or by uptake into mitochondria through the mitochondrial calcium uniporter. The entry of calcium ions through the mitochondrial calcium uniporter is an important step in mitochondrial ATP production (33, 34), which determine the excitation and relaxation cycle of the heart. Although calcium ions can be sourced from internal calcium stores such as endoplasmic and sarcoplasmic reticulum, voltage-gated Ca\textsuperscript{2+} channels are a rapid mechanism of Ca\textsuperscript{2+} influx and Ca\textsuperscript{2+} signalling in excitable cells (35).

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Ca\textsuperscript{2+} and ROS together play a role in the development of cardiac hypertrophy

ROS triggers the development of myocardial hypertrophy, extracellular matrix remodelling and cellular dysfunction. Various hypertrophic signalling kinases and transcription factors are stimulated by ROS (36). A consistent increase in ROS results in pathological cardiac remodelling and myocardial dysfunction (37). During mild oxidative stress, ROS at concentrations of \(<10\mu\text{M}\) can activate hypertrophic signalling kinases and transcription factors such as serine-threonine kinases, tyrosine kinases, Ca\textsuperscript{2+}/calmodulin-dependent protein kinases (CaMK), mitogen-activated protein kinases (MAPK) and nuclear factor of activated T cells (NFAT). ROS and Ca\textsuperscript{2+} signals are strongly linked to the development of cardiac hypertrophy. In cardiac tissue, an increase in mitochondrial calcium results in increased production of ROS from mitochondria (38). In addition, an increase in ROS from Q\textsubscript{o} site of complex III in the mitochondria mediates a further increase in intracellular Ca\textsuperscript{2+} via activation of the L-type Ca\textsuperscript{2+} channel (7, 9, 10, 12). This process known as “ROS-induced ROS-release” has been shown to activate hypertrophic signalling in cardiac myocytes (9, 12, 39, 40) (Fig 1) and is recognised as a mechanism for inducing oxidative stress in vascular smooth muscle (9, 41, 42) and in neurons (43, 44).

An increase in the diastolic Ca\textsuperscript{2+} is sufficient to contribute to phenotypic remodelling that leads to the development of hypertrophy (45-48). Alterations in calcium influx as a result of overexpression of the L-type Ca\textsuperscript{2+} channel is sufficient to initiate the development of hypertrophy (49). This leads to activation of calcium-calmodulin-CaMKII-HDAC (histone deacetylase) and calcium-calmodulin-calcineurin-NFAT that are recognised as two major Ca\textsuperscript{2+}-dependent hypertrophic signalling pathways (31).
Voltage gated calcium channels in the heart

Two types of voltage-gated calcium channels are observed in cardiac myocytes (50). The T-type calcium channel is expressed in embryonic heart but has limited expression in adult cardiac myocytes. It plays a significant role in controlling the pacemaker activity of the sinoatrial node. The abundant L-type Ca\(^{2+}\) channel is the main route for Ca\(^{2+}\) influx required for excitation-contraction coupling. Large conductance L-type Ca\(^{2+}\) channel is activated by high depolarization pulses allowing calcium ion entry into the cytosol (51). They possess selective dihydropyridine sensitivity, activated by the dihydropyridine Ca\(^{2+}\) channel agonist (S)-(−)-Bay K8644, and inhibited by dihydropyridine Ca\(^{2+}\) channel antagonists nisoldipine, nifedipine and nitrendipine. The L-type Ca\(^{2+}\) channel is a heterotetrameric polypeptide complex consisting of a pore forming α\(_1\) subunit (170–240kDa), an intracellular β subunit (55kDa), and extracellularly located disulphide linked α\(_{2}\)/δ subunit (170kDa) (52). The α\(_1\), α\(_{2}\)/δ and β\(_2\) subunits are mostly found in excitable cell types (53). The α\(_1\) subunit of the Ca\(^{2+}\) channel has four homologous motifs (I–IV), each composed of cytoplasmic NH\(_3\) and COOH terminal domains, six membrane spanning α-helices, termed S1 to S6, linked by variable length cytoplasmic loops (linkers) (54). The S5–S6 is the ion selective pore region, S4 is the voltage sensor segment, and binding site for drugs altering the function of the channel (55). To date, 10 genes have been identified coding α\(_1\) subunit proteins classified into four classes: Ca\(_{v}\)1.1 (α\(_{1}\)S), 1.2 (α\(_{1}\)C), 1.3 (α\(_{1}\)D) and 1.4 (α\(_{1}\)F). The α\(_{1}\)C subunit is mainly expressed in cardiac muscle, encoded by CACNA1C gene (53).

The β subunit is a supplementary subunit, which is strongly bound to cytoplasmic linkers between motifs I and II of cardiac α\(_{1}\)C, also known as the alpha interacting domain (AID). The β subunit plays a crucial role in controlling activation and inactivation kinetics and open probability of the channel. It is also important in the trafficking of the α\(_{1}\)c subunit into cell membrane (56). The N terminus of α\(_{1}\)C plays a role in channel voltage-gating (57–59) and protein kinase C (PKC) modulation (60). The long intracellular C terminus is responsible for Ca\(^{2+}\)- and voltage-dependent inactivation (52, 59), protein kinase A (PKA)-dependent modulation (although the exact site for PKA phosphorylation has been disputed in part because the complex beta-adrenergic signaling pathway is not yet fully understood.

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and also because heterogeneous expression systems used to study responses have resulted in conflicting findings) and transient anchoring of the protein in plasma membrane. Truncation of the C-terminus results in increased Ca\textsuperscript{2+}-currents relieving an inhibitory role of the tail (61, 62).

**Effect of altered redox state on the function of the cardiac L-type Ca\textsuperscript{2+} channel**

In a similar manner to heterologously expressed rabbit smooth muscle L-type Ca\textsuperscript{2+} channel and the human cardiac Na\textsuperscript{+} channel (63), the cardiac L-type Ca\textsuperscript{2+} channel (64-68) is responsive to changes in cellular redox state. In ferret and guinea pig ventricular myocytes, channel function can be enhanced by the thiol specific oxidising agent 5,5-dithio-bis[2-nitrobenzoic acid] (DTNB) and inhibited by the thiol reducing agent dithiothreitol (DTT) (69). Ferret ventricular myocytes exposed to 200 μM DTNB demonstrate increased calcium currents, whereas the addition of 1mM DTT inhibits the calcium currents (70). Acute exposure of the channel to H\textsubscript{2}O\textsubscript{2} or thiol oxidising agents results in increased channel activity, whereas subjecting the channel to thiol reducing agents or hypoxic conditions inhibits channel activity (65, 71-73). In human cardiomyocytes, glutathione is considered the main cytosolic redox buffer. Glutathione exists in a reduced state under normal physiological conditions. Glutathione reportedly modulates the redox state of the L-type Ca\textsuperscript{2+} channel and the Ca\textsubscript{v}1.2 channel protein has been shown to be persistently glutathionylated in ischemic human heart (68, 74). These studies demonstrate that the L-type Ca\textsuperscript{2+} channel protein is responsive to changes in the cellular redox state, and the subsequent alteration in channel function and calcium influx is sufficient to lead to the development of pathology associated with oxidative stress.

As stated, increased calcium influx through the L-type Ca\textsuperscript{2+} channel can contribute to pathology and the redox sensitivity of the L-type Ca\textsuperscript{2+} channel is especially important in ischemia/reperfusion injury. Activation of the channel by voltage or application of dihydropyridine agonists exacerbates “ROS-induced ROS-release” (75) and mitochondrial activity in cardiac myocytes (11, 37, 76) as well as in non-excitable cells, e.g. renal tubular cells (77). Since the auxiliary beta subunit tethers cytoskeletal proteins, it has been demonstrated that the channel can alter mitochondrial function as a result of

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movement of the beta subunit through cytoskeletal proteins following activation of the channel (37, 76, 78), in addition to the increase in intracellular calcium concentration (12, 79, 80).

**Role of cysteines in the altered redox state of proteins**

Cysteines are unique amongst the amino acids because they contain a reactive sulphhydryl or thiol (SH) group. Cross-linking (creation of a disulphide bond) between two cysteine residues may form between various parts of the same protein or between two separate polypeptide chains (81). The formation of a disulphide link is associated with the folding of a protein (and alteration in structure) and is assisted by the enzymes thiol-disulphide oxidoreductase and protein disulphide isomerise (68).

Cysteine residues are the preferred targets for redox or nitrosylation modification on proteins as free thiols can easily react with ROS or Reactive Nitrogen Species (RNS) (70). Changes in the redox state of the protein results in formation or disruption of disulphide bonds between the thiol/SH groups of cysteines. Protein sulphhydryls can be oxidised to protein disulphides, sulphenic acids and highly oxidised states such as sulphinic and sulphonic acid forms (82). In cysteine-rich proteins, the oxidation state of sulphhydryl groups of cysteine residues determines the structure and function of proteins. The reduction-oxidation state of proteins plays a significant role in ionic homeostasis, cellular host defence and cellular metabolism. Thiol oxidising and reducing agents are used to detect the redox sensitivity of proteins (68, 83-85)

**Understanding the structure-function relationship through redox modification of cysteines**

Intramolecular disulphide bonds preserve the structural integrity of cysteine-containing proteins, determining the folding and structural balance of ion channels, ion regulatory proteins, transporters and pumps (86). Under physiological reducing conditions, cytoplasmic cysteine residues rarely form disulphide bonds. In oxidative stress conditions disulphide bonds can be formed between adjacent cysteines in close proximity. While the structure of the Ca,1.2 protein (and the full native channel protein) have not been fully elucidated, many site-directed mutagenesis studies have been

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performed to understand the functional significance of cysteines in the altered redox state of ion channels (83), including the voltage-dependent potassium (Kv) channels (87), the ligand-gated cation channel P2X receptor (88), ryanodine receptor from skeletal (89) and cardiac muscle (90). Mutation of cysteines can disrupt the folding of ion channels e.g. inwardly rectifying K⁺ channels Kir2.1 (91), in addition to altering channel function. The substituted cysteine accessibility method introduces new cysteines into the amino acid sequence or substitutes the existing cysteines with non-reactive amino acids, usually adenine or serine, allowing the systematic investigation of the residues in a specific region of a protein, such as a membrane-spanning segment, residues forming a ligand binding site or ion pore. While cysteine substitution usually does not cause disruption of protein folding/expression, it may cause alterations in channel function that depend on the specific site of the substitution.

Cysteine substitution technique has an advantage over site directed mutagenesis, in that thiol modifying reagents can be used to investigate the role of cysteines in the structure and function of proteins. Functional changes could arise from structural changes on cysteine pairs in close proximity with minimal perturbation of the local or global protein structure. Thiol groups on the water accessible protein surface are ionized to thiolate anion, the most accessible to disulphide crosslinking or thiol modification. Therefore studying the reactivity of the engineered cysteine residues can reveal the relationship between structure and function of proteins, especially in the case of membrane proteins in different functional states, such as closed, open or desensitized/inactivated states. It is especially useful in the study of complex membrane proteins where high resolution structures are not available (83).

High resolution analysis of a three-dimensional protein structure is usually determined by X-ray crystallography or nuclear magnetic resonance (NMR) techniques. Circular dichroism spectroscopy is also used in protein structural studies, to identify small peptides, or specific parts of more complex proteins, such as α-helix, β-sheet and random coil, using their characteristic circular dichroism spectra. Although these methods have been successfully used for determining the structure, stability and ligand binding of many proteins, the size, solubility, and low expression level of a
protein can limit the use of these techniques for studying large molecular complexes, especially membrane proteins (92). The three-dimensional structure of many mammalian calcium channels remains unknown.

Fluorescent spectroscopy is an alternative method for examining protein structure detecting autofluorescent amino acids such as tryptophan/tyrosine or using specific fluorescent dyes such as 8-anilino-1-naphthalene sulphonate (ANS) (93), SYPRO Orange (94), 4,4′-bis (1-anilinonaphthalene 8-sulphonate) (bis-ANS) (95), or N-[4-(7-diethylamino-4-methyl-3-coumarinyl)phenyl]maleimide (CPM) (96). These fluorescent dyes either bind covalently to the thiol group of cysteines, α-amino group of the N-terminus, ε-amino group of lysines or non-covalently to the hydrophobic and electrostatic regions (97). A change in the three dimensional structure of the protein results in a change of the fluorescence spectra. Mass spectrometry and fluorescent studies using the thiol-specific probe monobromobimane have been used to identify redox-sensitive cysteines of RyR1. Point mutations of single cysteines or groups of cysteines have resulted in altered redox responses (89).

The thermal stability assay can provide further information on the stability of protein structures. It uses the protein melting method, where an increase in temperature results in the unfolding of proteins. This increases the available binding sites for the fluorescent dye, so the fluorescence signal increases. At higher temperatures fluorescence decreases when unfolded proteins gradually begin to aggregate. The midpoint of the temperature corresponding to the maximum fluorescence obtained on the unfolding of the protein induced by a high temperature is characteristic and denotes the stability of the protein (97, 98). The thermal stability assay is used to study the structural stability of proteins, coupling of functional domains, stability of ligand binding, and the communication between agonists and receptors. It can therefore identify free cysteines as possible targets for redox modification (96, 99-101).
A direct method for studying the redox modification of channel function

Reconstituting the heterologously expressed, then purified His-tagged channel protein into artificial liposomes has provided an opportunity to examine the function of a membrane protein at the molecular level (74, 102). It enables direct assessment of the effects of modification of cysteines on the channel function. The purity of channel protein is assessed by mass spectrometry. Contrary to other methods, such as heterologous expression systems or native cells there are only channel protein and lipid present, therefore the direct effect of redox modifications of cysteines can be observed in the absence of regulatory proteins.

The \( \alpha_{1c} \) subunit of the long N Terminal (LNT) isoform of the cardiac L-type Ca\(^{2+} \) channel protein (Ca\(_v\)1.2) contains 42 cysteines in total, 15 cysteines are extracellular, 8 are transmembrane and 19 are cytosolic (103). Under native conditions the interior of the cell is highly reduced and the cytoplasmic region of the channel is more likely to be targeted by redox agents under oxidative stress conditions. To examine this directly the reactive cysteines from different regions of the Ca\(_v\)1.2 channel protein were mutated to a serine or alanine and changes in open probability of single channel currents were assessed in the absence and presence of oxidised and reduced glutathione. Reconstitution of Ca\(_v\)1.2 protein alone is sufficient to form an ion conducting channel with single channel characteristics similar to native L-type calcium channels and sensitivity to the dihydropyridine blocker nisoldipine. (74) Mutation of cysteines in the intracellular Repeat I-II linker, the AID region (cysteines 519, 543 and 547 in the human LNT isoform) attenuated the effects of oxidising and reducing agents. More precisely C543 was critical for altering channel function in the presence of redox reagents and glutathione. It was also demonstrated that a point mutation of cysteine 543 to serine altered protein folding assessed using the thermal stability assay (104). These data confirm that redox modification of Ca\(_v\)1.2 channel protein alone is sufficient to alter structure and function of the channel under conditions of oxidative stress.
In physiological conditions the normal function of the Ca\textsubscript{1.2} subunit requires the auxiliary α2δ and β2 subunits. Since the β2 subunit modifies inactivation kinetics of the native L-type channel and the cysteine at 543 lies within the alpha interacting domain where the β2 subunit binds to the alpha subunit, redox modification of the Ca\textsubscript{1.2} subunit could alter the behaviour of the β2 subunit. Reactive oxygen species can alter the activity of many proteins including those closely associated with Ca\textsubscript{1.2} subunit and further influence the function of the voltage gated calcium channel in vivo.

**Conclusion and future directions based on identification of the critical cysteine**

Average lifespan is continuously increasing as well as the occurrence of age-associated diseases. As the Framingham Heart Study and the Baltimore Longitudinal Study on Aging demonstrated, the heart is particularly affected by aging (4, 5, 105, 106). The health burden associated with patients living with heart disease is increasing with the ageing population the development of new therapies for the prevention of cardiac hypertrophy and heart failure is critical to reduce the significant cost to individuals and to society.

Cardiac aging is associated with declined cardiac function, apoptosis, cardiac remodelling, making the heart more vulnerable to stress. Many studies demonstrated mitochondrial dysfunction and as a consequence altered ROS production in aging heart (107, 108).

The L-type calcium channel is the main route for calcium influx into cardiac muscle cells and is essential for excitation and contraction. It is well recognised that the native channel can respond to changes in cellular redox state and this mediates changes in intracellular calcium that are associated with the development of heart failure in the ischemic human heart. However it was unknown whether this was due to direct modification of thiol groups on the channel protein since contradictory responses have been reported in channels in cardiac myocytes or in expression systems. Recent studies have identified that the function of the purified ion conducting α\textsubscript{1C} subunit of the L-type Ca\textsuperscript{2+} channel is altered by thiol reducing and oxidising agents and the responsible cysteine has now been identified. During alterations in cellular redox state (hypoxia or oxidative...
stress), direct regulation of the Ca_{1.2} channel protein is sufficient to alter calcium influx and facilitate rapid physiological responses (64-67, 109). Since glutathionylation of Ca_{1.2} occurs in the ischemic human heart, and increased calcium influx through Ca_{1.2} is sufficient to induce hypertrophic growth, direct glutathionylation of Ca_{1.2} channel protein during reperfusion may contribute to the pathology associated with ischemic heart disease (74).

At the cellular level the L-type Ca^{2+} channel exists as a multimolecular complex, and its function is modified by auxiliary subunits and communication with many other regulatory proteins. Many proteins can modulate the function of the Ca_{1.2} subunit. The site of the critical cysteine on the Ca_{1.2} subunit is located at close proximity to the alpha-interacting domain where the β subunit binds to the pore forming α-subunit. Therefore further studies would be useful to determine how redox modification of cysteines can alter subunit interactions between the α_{1c} and β subunits, channel complex formation and compartmentalization. This may help us to further elucidate the mechanisms that lead to cardiac remodelling, and shape the development of therapy targeting the calcium channel.

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Disclosure

The authors declare no conflicts of interest.

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Figure legends

Fig 1. The cellular mechanisms leading to cardiac hypertrophy following an oxidative stress insult.
During reperfusion following ischemia the myocyte is exposed to an increase in hydrogen peroxide that increases calcium influx through the L-type Ca\textsuperscript{2+} channel as a result of oxidation of C543 on Ca\textsubscript{v}1.2 channel protein (9, 11, 74). The increased intracellular calcium concentration leads to further ROS production from the mitochondria as a result of increased mitochondrial calcium uptake (9, 39, 40) that maintains the L-type Ca\textsuperscript{2+} channel in an oxidised state. Increased levels of intracellular calcium ions and ROS simultaneously trigger many signal transduction pathways ultimately leading to hypertrophy.

Fig 2. Schematic diagram of the long N Terminal (LNT) isoform of Ca\textsubscript{v}1.2 channel protein with cysteines highlighted. The position of cysteines in extracellular loops (magenta), transmembrane regions (yellow), intracellular loops (red) and mutated cysteines (purple) are shown. Mutation of all 3 cysteines in loop I-II linker attenuates oxidative stress responses but C543 is critical to mediating alterations in channel function following an oxidative stress insult (see text for explanation).