World Journal of Cardiology

Online Submissions: http://www.wjgnet.com/1949-8462office
wjc@wjgnet.com
doi:10.4330/wjc.v3.i7.215

World J Cardiol 2011 July 26; 3(7): 215-218
ISSN 1949-8462 (online)

Calcium channels and iron uptake into the heart

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Author contributions: All authors contributed equally to this work.
Supported by Thailand Research Fund grants RTA5280006 (Chattipakorn N), BRG5480003 (Chattipakorn S), the National Research Council of Thailand (Chattipakorn N), and the Thailand Research Fund Royal Golden Jubilee project (Kumfu S and Chattipakorn N)
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Received: April 19, 2011 Revised: July 4, 2011
Accepted: July 11, 2011
Published online: July 26, 2011

Abstract

Iron overload can lead to iron deposits in many tissues, particularly in the heart. It has also been shown to be associated with elevated oxidative stress in tissues. Elevated cardiac iron deposits can lead to iron overload cardiomyopathy, a condition which provokes mortality due to heart failure in iron-overloaded patients. Currently, the mechanism of iron uptake into cardiomyocytes is still not clearly understood. Growing evidence suggests L-type Ca^{2+} channels (LTCCs) as a possible pathway for ferrous iron (Fe^{2+}) uptake into cardiomyocytes under iron overload conditions. Nevertheless, controversy still exists since some findings on pharmacological interventions and those using different cell types do not support LTCC's role as a portal for iron uptake in cardiac cells. Recently, T-type Ca^{2+} channels (TTCC) have been shown to play an important role in the diseased heart. Although TTCC and iron uptake in cardiomyocytes has not been investigated greatly, a recent finding indicated that TTCC could be an important portal in thalassemic hearts. In this review, comprehensive findings collected from previous studies as well as a discussion of the controversy regarding iron uptake mechanisms into cardiomyocytes *via* calcium channels are presented with the hope that understanding the cellular iron uptake mechanism in cardiomyocytes will lead to improved treatment and prevention strategies, particularly in iron-overloaded patients.

Key words: Cardiomyocytes; L-type calcium channel; T-type calcium channels; Iron overload; Thalassemia

Peer reviewers: Morten Grunnet, PhD, Professor, Head of Drug Discovery Portfolio Management, NeuroSearch A/S, 2750 Ballerup, Denmark; Mohamed Chahine, PhD, Professeur Titulaire, Le Centre de Recherche Université Laval Robert-Giffard, Local F-6539, 2601 chemin de la Canardière, Québec G1J 2G3, Canada

Chattipakorn N, Kumfu S, Fucharoen S, Chattipakorn S. Calcium channels and iron uptake into the heart. *World J Cardiol* 2011; 3(7): 215-218 Available from: URL: http://www.wjgnet.com/1949-8462/full/v3/i7/215.htm DOI: http://dx.doi.org/10.4330/wjc.v3.i7.215

INTRODUCTION

Iron (Fe) is an essential element for all living organisms and plays a central role in many Fe-containing proteins such as in iron storage proteins (ferritin and hemosiderin), energy metabolism (cytochromes, mitochondrial aconitase and Fe-S proteins of the electron transport chain), cellular respiration (hemoglobin and myoglobin), and DNA synthesis (ribonucleotide reductase)[1-3]. However, under iron overload conditions the regulatory mechanism which keeps the balance between iron uptake

and iron excretion could be disrupted, causing an elevation of non-transferrin bound iron (NTBI) in the plasma of iron-overloaded patients[4,5]. NTBI is toxic and participates in the production of harmful hydroxyl radicals, which could cause severe cellular damage and organ dysfunction[1,2,6,7].

An excess of plasma iron can lead to iron accumulation in many organs including the heart[5]. Excessive iron accumulation in the heart can cause cardiac cellular damage known as iron-overload cardiomyopathy. This cardiac complication causes 71% of all deaths in thalassemia major patients[8]. Although iron chelation therapy is widely used for treating iron overload patients, iron overload cardiomyopathy is still the most common cause of mortality in these patients[9,10]. Even though the fundamental mechanisms for excessive iron uptake in the heart have been investigated for decades, the precise mechanism underlying cardiomyocyte dysfunction induced by iron overload is not clearly understood. Although several NTBI transporters have been proposed and are responsible for cellular iron uptake, recent evidence suggests that calcium channels may play an important role as a portal for cardiac iron uptake[11].

In this review, the role of L-type Ca^{2+} channels (LTCC) as well as T-type Ca^{2+} channels (TTCC) as iron transporters into the heart are presented. The consistent findings as well as discrepancies of results among various studies on iron uptake into cardiomyocytes *via* these calcium channels under various conditions are comprehensively reviewed and discussed.

LTCCS AS A PORTAL FOR IRON UPTAKE INTO CARDIOMYOCYTES

The L-type Ca^{2+} channel is a voltage-gated ion channel that plays a central role in cardiac and smooth muscle contraction[12]. LTCCs are heterotetrameric polypeptide complexes that are composed of $\alpha1$, $\alpha2/\delta$, β, and, in some tissues, γ subunits[12]. The Ca^{2+} channel $\alpha1$ subunit (170-240 ku) is organized into four homologous motifs (I -IV), with six transmembrane segments (S1-S6)[12]. Recently, 10 $\alpha1$ subunit genes have been identified including $Ca_v1.1$ ($\alpha1S$), 1.2 ($\alpha1C$), 1.3 ($\alpha1D$), 1.4 ($\alpha1F$), $Ca_v2.1$ ($\alpha1A$), 2.2 ($\alpha1B$), 2.3 ($\alpha1E$), $Ca_v3.1$ ($\alpha1G$), 3.2 ($\alpha1H$), and 3.3 ($\alpha1I$). For LTCCs, these can be divided into 4 classes: $Ca_v1.1$ ($\alpha1S$), 1.2 ($\alpha1C$), 1.3 ($\alpha1D$), and 1.4 ($\alpha1F$). In cardiac muscles, only the $\alpha1C$ (dihydropyridine-sensitive) subunit is expressed in high levels and is also called a high-voltage-activated channel[12]. LTCCs can be found in the heart and are primarily used for Ca^{2+} transport as well as playing an important role in the electrical activity of the heart. However, previous studies have shown that LTCCs can also transport other divalent cations including Fe^{2+}[13-15].

Several findings have been shown to support the role of LTCC in myocardial iron transport[11,15]. A study in an iron loaded perfused rat heart showed that iron uptake was increased by the LTCC agonist, Bay K 8644 and iron uptake was inhibited by the LTCC blocker, nifedipine[15]. Oudit *et al*[16] demonstrated that treatments with LTCC blockers such as amlodipine and verapamil could lead to the inhibition of LTCC current in cardiomyocytes, reduced myocardial iron accumulation, decreased oxidative stress and improved survival in iron-loaded mice. In addition, iron overloaded transgenic mice with cardiac-specific overexpression of LTCC were shown to have increased myocardial iron accumulation and oxidative stress, resulting in impaired cardiac function in comparison with control mice[16]. Furthermore, since the LTCC does not contain iron responsive elements (IREs) in the LTCC mRNA, it is not regulated by cellular iron levels under an iron overload condition. As a result, L-type Ca^{2+} currents were not decreased in iron overload conditions[16], confirming that the expression of LTCC was not regulated by the IRE. Furthermore, it has been shown in iron overloaded rats that the LTCC blocker diazepam could reduce mortality from iron overload without inhibition of iron absorption or urinary iron excretion[17].

In addition to the heart, a previous study also demonstrated that LTCC blockers verapamil and amlodipine did not decrease iron accumulation in the liver of mice with iron overload, and hypothesized that this was due to the fact that hepatocytes express minimal levels of LTCC[16]. However, a recent study by Ludwiczek and colleagues demonstrated that the LTCC blocker nifedipine could reduce iron accumulation in the liver of wild-type mice, but had no effect in divalent metal transporter 1 (DMT1) deficient mice, suggesting that this effect of nifedipine-mediated modulation of iron transport is *via* DMT1[18]. Nevertheless, these findings suggest that nifedipine could possibly be beneficial in iron overload cardiomyopathy.

DISCREPANCIES IN FINDINGS ON IRON UPTAKE INTO CARDIOMYOCYTES *VIA* LTCC

It is important to realize that not all reports regarding the mechanisms of iron uptake *via* LTCC are consistent. Despite strong evidence supporting the role of LTCC as a route for NTBI transport in the heart, Parkes and colleagues demonstrated otherwise[19]. In cultured rat neonatal myocytes, they demonstrated that LTCC blockers (nifedipine, verapamil, and diltiazem) did not alter iron uptake in these cells[19]. Our recent findings also demonstrated that the LTCC blocker verapamil could not prevent iron uptake into cultured adult mouse cardiomyocytes[20].

Several reasons to explain these inconsistent results may be drawn from previous reports. Most studies that support the role of LTCC for iron uptake in cardiomyocytes used freshly prepared cardiomyocytes taken from isolated perfused hearts[15] or *in vivo*[16]. However, a report that failed to show the role of LTCC in iron uptake into cardiomyocytes used cultured cardiomyocytes[19,20].

In cultured cardiomyocytes, it is possible that LTCC

may not fully develop compared with isolated cardiomyocytes obtained from the heart. If fully developed, it is also possible that the LTCC in cultured cardiomyocytes may not function properly. Furthermore, the ages of cultured cardiomyocytes could have played a role in this discrepancy.

In the light of these inconsistent findings, it is possible that cardiomyocytes obtained from different methods may have different cellular characteristics and properties. All of these proposed hypotheses have not been tested and will need to be further investigated to elucidate the definite mechanism of iron uptake into the heart and resolve these existing discrepancies.

TTCC AS A PORTAL FOR IRON UPTAKE INTO CARDIOMYOCYTES

TTCC have three isoforms: $Ca_v3.1$ (α1G), 3.2 (α1H), and 3.3 (α1I) that are localized to the brain, kidney, and heart and are also called low-voltage-activated channels[21]. It has been shown that only $Ca_v3.1$ and $Ca_v3.2$ are expressed in the heart[21]. TTCCs have been reported to be functionally expressed only in embryonic hearts and disappear in adults[22]. TTCC can be found abundantly only in sinoatrial pacemaker cells and Purkinje fibers of many species in adult hearts and are important for the maintenance of pacemaker activity[21,23]. However, TTCC currents and expression have been demonstrated to reappear and play an important pathological role in diseased hearts with conditions such as ventricular hypertrophy[21,24,25] and post-myocardial infarction[26]. The increased TTCC expression has been shown to contribute to the progression of heart failure[21].

Growing evidence indicates that TTCC blockers could be beneficial in diseased hearts. Recently, Horiba and colleagues demonstrated that the blockade of Ca^{2+} entry into cardiomyocytes *via* TTCC using the TTCC blocker efonidipine could block signal transduction involved in cardiac hypertrophy[27]. In addition, a study in a mouse model of dilated cardiomyopathy has shown that a TTCC blocker could restore the resting membrane potential, and reduce the number of premature ventricular contractions and ventricular tachycardia, thus reducing the incidence of sudden death in these mice[28]. These findings suggest that TTCC blockade may be potentially useful for the prevention of sudden death in patients with heart failure[28]. It is known that iron overload conditions can lead to increased iron uptake into cardiomyocytes, resulting in cardiac hypertrophy and failure[29-32]. However, it is not known if TTCC blockers could be cardioprotective in this type of cardiomyopathy.

Recently, our study using cultured cardiomyocytes taken from the heart of thalassemic mice demonstrated that intracellular iron accumulation in cultured ventricular myocytes of thalassemic mice was significantly higher than in wild type (WT) cells[20]. These findings suggest that thalassemic cardiomyocytes could have pathways which can greatly uptake iron into the cells more than that in WT cells. In addition, under an iron overloaded condition, our results demonstrated that the TTCC blocker, efonidipine, could prevent iron uptake into cultured thalassemic cardiomyocytes[20]. Although efonidipine is not a selective TTCC blocker and could also block LTCC, its efficacy in blocking TTCC is greater than that of LTCC[21]. In that study, since verapamil could not prevent iron uptake when efonidipine could, these findings suggested that TTCC could play a significant role in iron uptake into cardiomyocytes in this thalassemic cardiomyocyte model[20]. Moreover, our microarray data demonstrated that the TTCC genes were up-regulated in thalassemic hearts, which is well correlated with the iron uptake results, suggesting that TTCCs could play an important role in iron uptake in thalassemic hearts, and that their re-expression could be due to the pathological state of a thalassemic heart itself or from the iron-overloaded condition, or both.

Since iron overload patients can develop cardiomyopathy and heart failure[33-35], it is important that the association between iron overload, TTCC expression/function and cardiac complications be determined. Future studies in both basic and clinical research are needed to warrant the clinical usefulness of TTCC blockers in the prevention and treatment of iron overload cardiomyopathy particularly in thalassemia patients.

CONCLUSION

Iron overload is a serious and fatal complication in many diseases including iron-overload cardiomyopathy in thalassemia patients. Although pathways for cellular iron uptake have been investigated for many decades, its mechanism is still not clearly understood. In the past few years, findings regarding new possible pathways for cellular iron uptake have been suspected, including LTCC and TTCC. However, their definite roles as iron transporters in cardiomyocytes are still debated. Understanding the mechanism by which iron enters cardiac cells is very important, since it will provide us with the knowledge to be used in developing better treatment and prevention strategies in iron overloaded patients.

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S- Editor Cheng JX **L- Editor** O'Neill M **E- Editor** Zheng XM