Calcium channels and iron uptake into the heart

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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 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.

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). 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\textsuperscript{[4,5]}. NTBI is toxic and participates in the production of harmful hydroxyl radicals, which could cause severe cellular damage and organ dysfunction\textsuperscript{[6,7]}. An excess of plasma iron can lead to iron accumulation in many organs including the heart\textsuperscript{[8]}. 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\textsuperscript{[9]}. 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\textsuperscript{[8,10]}. 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\textsuperscript{[11]}. In this review, the role of L-type Ca\textsuperscript{2+} channels (LTCC) as well as T-type Ca\textsuperscript{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\textsuperscript{2+} channel is a voltage-gated ion channel that plays a central role in cardiac and smooth muscle contraction\textsuperscript{[12]}. LTCCs are heterotetrameric polypeptide complexes that are composed of α1, α2βδ, β, and, in some tissues, γ subunits\textsuperscript{[13]}. The Ca\textsuperscript{2+} channel α1 subunit (170-240 kD) is organized into four homologous motifs (I–IV), with six transmembrane segments (S1–S6)\textsuperscript{[14]}. Recently, 10 α1 subunit genes have been identified including Ca-1.1 (α1S), 1.2 (α1C), 1.3 (α1D), 1.4 (α1F), Ca-2.1 (α1A), 2.2 (α1B), 2.3 (α1E), Ca-3.1 (α1G), 3.2 (α1H), and 3.3 (α1I). For LTCCs, these can be divided into 4 classes: Ca-1.1 (α1S), 1.2 (α1C), 1.3 (α1D), and 1.4 (α1F). In cardiac muscles, only the α1C (dihydropyridine-sensitive) subunit is expressed in high levels and is also called a high-voltage-activated channel\textsuperscript{[12]}. LTCCs can be found in the heart and are primarily used for Ca\textsuperscript{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\textsuperscript{3+}\textsuperscript{[13-15]}. Several findings have been shown to support the role of LTCC in myocardial iron transport\textsuperscript{[11,14]}. 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\textsuperscript{[15]}. Oudit et al\textsuperscript{[14]} 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\textsuperscript{[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\textsuperscript{2+} currents were not decreased in iron overload conditions\textsuperscript{[14]}, 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\textsuperscript{[17]}.

In addition to the heart, a previous study also demonstrated that LTCC blockers verapamil and amiodipine 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\textsuperscript{[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\textsuperscript{[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\textsuperscript{[19]}. In cultured rat neonatal myocytes, they demonstrated that LTCC blockers (nifedipine, verapamil, and diltiazem) did not alter iron uptake in these cells\textsuperscript{[19]}. Our recent findings also demonstrated that the LTCC blocker verapamil could not prevent iron uptake into cultured adult mouse cardiomyocytes\textsuperscript{[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\textsuperscript{[18]} or in vivo\textsuperscript{[20]}. However, a report that failed to show the role of LTCC in iron uptake into cardiomyocytes used cultured cardiomyocytes\textsuperscript{[15,21]}.

In cultured cardiomyocytes, it is possible that LTCC
Disorders of iron metabolism.
Rowley DA, Griffiths E, Halliwell B. Low- 
Ponka P, Richardson DR. Iron trafficking in the 
cells more than that in 
thalassemic cardiomyocytes could have played a role in this 
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 re-
solve these existing discrepancies.

**TTCC AS A PORTAL FOR IRON UPTAKE INTO CARDIOMYOCYTES**

TTCC have three isoforms: Ca3.1 (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 Ca3.1 and Ca3.2 are expressed in 
the heart[21]. TTCCs have been reported to be function-
ally expressed only in embryonic hearts and disappear in 
adults[24]. TTCC can be found abundantly only in sino-
atrial 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 ex-
pression have been demonstrated to reappear and play an 
important pathological role in diseased hearts with con-
ditions such as ventricular hypertrophy[21,24,25] and post-
myocardial infarction[30]. The increased TTCC expression 
have 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 Ca2+
entry into cardiomyocytes via TTCC using the TTCC 
blocker efonidipine could block signal transduction in-
volved 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 po-
tential, 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[29]. It is known that iron overload condi-
tions can lead to increased iron uptake into cardiomyo-
cytes, resulting in cardiac hypertrophy and failure[25,26,30]. However, it is not known if TTCC blockers could be 
antiarrhythmic 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[24]. 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, efoni-
dipine, 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 cardiomyo-
cytes in this thalassemic cardiomyocyte model[20]. More-
over, our microarray data demonstrated that the TTCC 
genesis 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 cardiomyop-
athy and heart failure[31,32], it is important that the associa-
tion 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 preven-
tion and treatment of iron overload cardiomyopathy par-
ticularly in thalassemia patients.

**CONCLUSION**

Iron overload is a serious and fatal complication in many 
diseases including iron-overload cardiomyopathy in thal-
assemia patients. Although pathways for cellular iron up-
take have been investigated for many decades, its mecha-
nism 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 car-
diomyocytes are still debated. Understanding the mecha-
nism 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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