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# *Calcium homeostasis and its dysregulation in ischemia-reperfusion injury: A study*

**Yunlong Zhong, Jiaji Zhang, Shoutian Li***

*School of Forensic Medicine, Zunyi Medical University, Zunyi, Guizhou, 56300, China*
**Corresponding author*

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***Abstract:*** Patients with acute ischemic cerebrovascular and cardiovascular disease develop ischemia-reperfusion injury (IRI) after normal blood flow is restored to the affected area. This phenomenon is related to calcium homeostasis. Homeostasis of intracellular calcium is essential for maintaining cellular vital activities. This paper is divided into five parts based on the actual scenario, and analyzes in detail the research progress of calcium homeostasis and its dysregulation in ischemia-reperfusion injury. Firstly, the regulatory mechanism of calcium ion homeostasis was analyzed, and then the effect of ischemia-reperfusion injury on calcium homeostasis was discussed. Then, the treatment strategies for calcium homeostasis dysregulation and regulation of calcium homeostasis in ischemia-reperfusion injury were elucidated. Finally, this article summarizes this topic and aims to provide reference materials for researchers in the field.

Ischemia-reperfusion injury (IRI) is a common and severe type of tissue damage that generally occurs during the ischemia-reperfusion process. The onset of the disease leads to phenomena such as insufficient tissue oxygen supply and accumulation of metabolic products, adversely affecting the patient's health. Calcium ions play a crucial role in intracellular signal transmission and regulation, maintaining normal cellular functions. The homeostasis of intracellular calcium is vital for normal physiological processes. The occurrence of IRI disrupts intracellular calcium homeostasis, leading to an imbalance and triggering a series of pathological physiological reactions. During ischemia, the decline in intracellular ATP levels results in decreased function of the cell membrane calcium pump, causing an increase in free calcium concentration in the cytoplasm and mitochondria. Additionally, it's worth noting that hypoxia and acidosis can also cause extracellular calcium to enter the cell, further increasing intracellular calcium concentration. High calcium concentrations activate various enzymes in the cytoplasm, leading to protein damage and cell apoptosis. During reperfusion, the high concentration of oxygen supply and the accumulation of metabolic products damage the integrity of the cell membrane, increasing its permeability. In this context, extracellular calcium ions freely enter the cell, further increasing intracellular calcium concentration. High calcium activates intracellular signaling pathways, exacerbating tissue damage. Moreover, high calcium concentrations can also cause mitochondrial dysfunction, leading to loss of mitochondrial membrane potential and increased ROS production. Based on these insights, this article provides an in-depth analysis of the research progress on calcium homeostasis and its dysregulation in ischemia-reperfusion injury, presented as follows.

## 1. Mechanisms of Calcium Ion Homeostasis Regulation

### 1.1. Sources and Movement of Calcium Ions within Cells

One source of intracellular calcium ions is from internal storage, making it an important source of intracellular calcium ions, specifically from the endoplasmic reticulum (ER) and mitochondria. The ER serves as the intracellular calcium store, where calcium ions from the cytoplasm are loaded into the ER lumen by the calcium pump SERCA. Simultaneously, the ER contains calcium ion channels IP3R and RyR. When cell release agents bind to their respective receptors, it leads to the opening of these channels, releasing calcium ions from the ER lumen into the cytoplasm. Additionally, mitochondria contain a high concentration of free calcium ions, primarily maintained by mitochondrial calcium channels MCU and NCX. Mitochondria participate in energy metabolism and regulate cell death by maintaining intracellular calcium ion levels. There are various calcium channel proteins on the cell membrane. VGCCs are usually in a closed state; they open when there is a change in cell membrane potential, allowing extracellular calcium ions to enter the cell. LGCCs open/close due to the action of extracellular signaling molecules. Transporters extrude intracellular calcium ions from the cell through ATPase-driven processes, regulating the concentration of intracellular calcium ions. In the cell, the dynamic balance of calcium ion concentration is primarily achieved through the combined action of calcium pumps, calcium channels, and calcium-binding proteins. Calcium pumps reload calcium ions from the cytoplasm into the ER or extrude them from mitochondria through NCX, a process that consumes energy. In contrast, calcium channels regulate the influx and efflux of calcium ions, with their activity being controlled by various factors. Additionally, it's important to note that calcium-binding proteins are also a crucial component in the regulation of intracellular calcium ion flow. Calcium-binding proteins can form complexes with calcium ions to regulate calcium ion-related cellular signal transduction[1].

### 1.2. Key Factors and Regulatory Pathways in Intracellular Calcium Ion Homeostasis

Various types of calcium ion channels exist on the cell membrane. Voltage-gated calcium ion channels open/close based on the difference in electric potential inside and outside the cell. Common types include voltage-gated sodium-calcium exchange channels and voltage-gated calcium channels. Ligand-gated calcium channels are primarily operated through ligand binding, with common types including glutamate receptors and calmodulin receptors. Literature indicates that calcium/sodium exchange pumps maintain low intracellular calcium ion concentration in a resting state by actively transporting intracellular calcium ions in exchange for external sodium ions. Calcium/proton exchange pumps primarily exchange intracellular calcium ions with extracellular protons to maintain stable intracellular calcium ion concentrations. Studies show that when cells are stimulated externally, calcium ion channels open, leading to changes in intracellular and extracellular calcium ion concentrations. Intracellular organelles like the endoplasmic reticulum and mitochondria can also release calcium ions, increasing the concentration of free intracellular calcium ions. Free calcium ions can interact with calcium-binding proteins to form calcium-binding complexes, mediating the transmission of calcium signals. Calcium-binding complexes can regulate a range of enzyme activities within the cell, activating or inhibiting protein kinases, phosphatases, and other enzymes, affecting intracellular biological processes. Calcium ion pumps use energy dissipation mechanisms to actively extrude intracellular calcium ions out of the cell, thereby restoring the concentration difference of calcium ions inside and outside the cell. Calcium-binding proteins, through calcium dissociation action, promote the binding of free calcium ions to other molecules, reducing the concentration of free intracellular calcium ions[2].

## 2. The Impact of Ischemia-Reperfusion Injury on Calcium Homeostasis

### 2.1. The Impact of Ischemia on Calcium Homeostasis

Literature indicates that under normal physiological conditions, the concentration of calcium ions inside and outside the cell is dynamically balanced, maintaining normal cellular functions. However, it is noteworthy that when tissue suffers ischemia, this balance of calcium ions is disrupted, leading to an increase in intracellular calcium concentration. The reduced activity of the cell membrane calcium pumps is a primary cause for the rise in intracellular calcium concentration during ischemia. Calcium pumps transport calcium ions from inside the cell to the outside, effectively maintaining calcium ion balance. During ischemia, due to insufficient energy supply, reduced activity of calcium pumps in the cell membrane leads to an accumulation of intracellular calcium ions. High concentrations of intracellular calcium ions can trigger the opening of mitochondrial calcium ion carriers, leading to an increase in mitochondrial calcium concentration, causing mitochondrial permeability transition, release of cytochrome c and other apoptotic factors, and leading to cell apoptosis or necrosis. Excess intracellular calcium ions can activate intracellular enzymes, causing subcellular structural damage and cellular dysfunction[3].

### 2.2. The Impact of Reperfusion on Calcium Homeostasis

During the ischemia-reperfusion process, changes in intracellular and extracellular calcium ion concentrations can activate various calcium-related signal transduction pathways. Activation of these pathways can lead to numerous cell function and injury-related biological effects. The increase in intracellular calcium ion concentration may also lead to an accumulation of calcium ions in mitochondria, triggering the opening of mitochondrial permeability transition pores (MPTP). This situation can lead to leakage of mitochondrial proteins, loss of membrane potential, and inhibition of ATP synthesis, resulting in cell energy depletion and cell death. Additionally, it is worth mentioning that during reperfusion, an increase in calcium ion concentration can also affect the function of vascular endothelial cells. Excessively high calcium ion concentrations can lead to a reduction in intracellular NO synthesis, causing vascular constriction and spasm.

## 3. Calcium Homeostasis Imbalance in Ischemia-Reperfusion Injury

### 3.1. Calcium Homeostasis Changes During Ischemia

#### 3.1.1. Increased Intracellular Calcium Ion Influx Due to Ischemia

ATP is one of the key substances that maintain the balance of calcium ions inside and outside the cell. When cellular ATP levels decrease, the activity of ATP-dependent calcium pumps in the cell diminishes, leading to an increase in intracellular calcium ion concentration. During ischemia, a decrease in intracellular pH value results in an increased probability of opening the cell membrane calcium channels, causing an increased inflow of high-level calcium ions into the cell. Ischemia-reperfusion increases intracellular oxidative stress, leading to an increase in the generation of oxygen free radicals. These radicals interact with intracellular calcium ion channels, eventually leading to an increase in calcium channel permeability and calcium ion influx. The increase in calcium ion influx caused by ischemia can lead to various adverse consequences, including cell damage due to high intracellular calcium ion concentration. Excessively high intracellular calcium ion concentration can also activate various enzymes and proteases, destroying intracellular structures and functions. Literature confirms that the increase in calcium ion influx due to ischemia plays a significant role in

ischemia-reperfusion injury. Ischemia-reperfusion activates L-type voltage-gated calcium channels, further increasing intracellular calcium ion concentration and causing cardiomyocyte death[4].

#### 3.1.2. Changes in Calcium Ion Pump and Channel Functions

Calcium ion pumps include cytoplasmic and mitochondrial calcium ion pumps. During ischemia, the activity level of cytoplasmic calcium ion pumps decreases, preventing the extrusion of excess intracellular calcium ions. In this situation, the continuous rise in intracellular calcium ion concentration activates various enzyme systems, causing cell damage and death. Additionally, impairment of mitochondrial calcium ion pumps leads to accumulation of calcium ions in mitochondria, causing mitochondrial dysfunction and apoptosis. During ischemia, the state of various calcium ion channels in the cell membrane also changes. An increase in the production of reactive oxygen species (ROS) can activate various calcium ion channels, such as transmembrane phosphatase family channels and stress-sensitive calcium ion channels, thereby increasing intracellular calcium ion concentration levels. Furthermore, ischemia disrupts the ion balance of the cell membrane, increasing the activity of voltage-gated calcium ion channels and causing an influx of calcium ions.

### 3.2. Calcium Homeostasis Changes During Reperfusion

#### 3.2.1. Further Increase in Calcium Ion Influx

Calcium ion influx in ischemia-reperfusion injury mainly occurs through two pathways: the increased opening of voltage-gated Ca2+ channels and the activation of intracellular Ca2+ release mechanisms. Literature confirms that during reperfusion, changes in cell membrane potential caused by ischemia increase the probability of opening voltage-gated Ca2+ channels, forcing more Ca2+ into the cell. In myocardial ischemia-reperfusion injury, the probability of opening L-type voltage-gated Ca2+ channels significantly increases, leading to increased Ca2+ influx. Additionally, ischemia-reperfusion injury can activate intracellular calcium mobilization mechanisms, causing the release of stored Ca2+ from within the cell. Specifically, ischemia-reperfusion injury leads to a decrease in intracellular ATP levels and changes in intracellular pH, activating calcium release channels in the cytoplasm or mitochondria, resulting in the release of intracellular Ca2+ into the cytoplasm[5].

#### 3.2.2. Activation of Calcium Ion Release Channels

Calcium ion release channels primarily include the sarcoplasmic reticulum calcium release channel (RyR) and mitochondrial calcium ion channels. Literature confirms that the increase in intracellular calcium ion (Ca2+) concentration caused by ischemia leads to the activation of calcium ion release channels during reperfusion, causing calcium homeostasis imbalance. During ischemia, a decrease in cellular ATP levels and mitochondrial dysfunction lead to the activation of mitochondrial calcium ion channels. Activation of these channels increases mitochondrial calcium ion concentration, inducing uncoupling protein phosphorylation, and increasing mitochondrial calcium ion release. Additionally, cellular acidification caused by ischemia during reperfusion can also promote the activation of sarcoplasmic reticulum calcium release channels. Literature shows that under acidic conditions, the probability of opening RyR channels increases, and the degree of calcium ion release is also amplified, leading to calcium homeostasis imbalance[6].

### 3.3. The Impact of Calcium Homeostasis Imbalance on Cell Damage

#### 3.3.1. Cytotoxic Effects Caused by Calcium Ion Overload

Literature indicates that transient ischemia leads to increased ATP consumption within cells, inhibiting the activity of Ca2+-ATPase and reducing the capacity for calcium ion extrusion. Ischemia-reperfusion leads to the generation of a large number of oxygen free radicals, which inhibit the activity of Ca2+-ATPase. Inability to promptly extrude calcium ions results in an increased intracellular calcium ion concentration and cell death. Calcium ion overload leads to the accumulation of calcium ions in mitochondria, disrupting the mitochondrial electrochemical gradient and membrane permeability. During ischemia-reperfusion, the restoration of the ER environment is severely disrupted, calcium ion concentration rises, ER membrane-related proteins abnormally fold, activating cell apoptosis signaling pathways and inducing cell death. In IRI, a large number of cells release damage-associated molecular patterns, which activate surrounding immune cells, causing an inflammatory response and exacerbating cell damage[7].

#### 3.3.2. Changes in the Function of Calcium Ion-Regulating Proteins

Literature shows that calcium homeostasis imbalance leads to an increase in intracellular calcium ion concentration. During ischemia, reduced cellular ATP levels limit the capacity of calcium ion pumps, impeding timely calcium ion extrusion. Simultaneously, ischemia also causes the release of a large amount of calcium ions from intracellular stores, increasing intracellular calcium ion concentration. Excess calcium ions can activate a series of enzymes, and excessive enzyme activation can lead to damage to the cell's DNA, RNA, and proteins. Ischemia-reperfusion-induced calcium ion entry into mitochondria causes changes in the mitochondrial environment, leading to a transformation in mitochondrial inner membrane permeability and mitochondrial dysfunction, resulting in reduced ATP synthesis and activation of mitochondrial cell death mechanisms.

## 4. Therapeutic Strategies for Calcium Homeostasis Regulation in Ischemia-Reperfusion Injury

### 4.1. Application of Calcium Channel Blockers

Calcium channel blockers are a class of drugs that can block or weaken the opening of calcium channels on cell membranes, reducing calcium ion influx. By using calcium channel blockers, the severity of ischemia-reperfusion injury can be mitigated as part of the treatment strategy. Currently, widely used calcium channel blockers include non-dihydropyridine calcium antagonists (such as procaine and lidocaine), dihydropyridine calcium antagonists (such as nifedipine and diltiazem), and drugs like aripiprazole. These medications can target various calcium channels to exhibit therapeutic effects. Literature reports the protective role of procaine in ischemia-reperfusion injury, which works by inhibiting the increase of intracellular calcium ions, reducing oxidative stress and inflammatory responses, thus protecting cardiac tissues[8].

### 4.2. Application of Calcium Pump Activators

Calcium ion regulation plays a crucial role in ischemia-reperfusion injury, primarily because abnormal increases in calcium ions can lead to a series of pathological responses, such as cell apoptosis, oxidative stress, and inflammatory reactions. Calcium pumps are a class of transmembrane transport proteins capable of transporting intracellular calcium ions to the outside of the cell through energy consumption, effectively maintaining the balance of calcium ion concentration inside and outside the cell. Activating calcium pumps can comprehensively reduce intracellular calcium ion

levels, thereby alleviating calcium-ion-mediated cell damage.

## 5. Conclusion

In summary, this article comprehensively discusses the research progress on calcium homeostasis and its dysregulation in ischemia-reperfusion injury. It analyzes the relationship between calcium metabolism and ischemia-reperfusion, aiming to provide insights for the clinical development of calcium channel antagonist drugs and treatments for ischemia-reperfusion injuries. Currently, there is still debate in the clinical understanding of the complex biological processes involved in calcium homeostasis and the destabilization following ischemia-reperfusion injury. As the academic community deepens its understanding, this has sparked interest among scholars in researching calcium-related knowledge and uncovering hidden mechanisms. The article summarizes and analyzes new evidence of calcium signaling in cardiovascular and cerebrovascular diseases, with the objective of drawing attention to this issue among relevant professionals. It delves into the mechanisms of action of calcium signaling in secondary reperfusion injury, highlighting its significance in this context.

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