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REVIEW ARTICLE

Modes of Calcium Regulation in Ischemic Neuron

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Abstract Calcium (Ca^{2+}) dysregulation is a major catalytic event. Ca^{2+} dysregulation leads to neuronal cell death and brain damage result in cerebral ischemia. Neurons are unable in maintaining calcium homeostasis. Ca^{2+} homeostasis imbalance results in increased calcium influx and impaired calcium extrusion across the plasma membrane. Ca^{2+} dysregulation is mediated by different cellular and biochemical mechanism, which leads to neuronal loss resulting stroke/cerebral ischemia. A better understanding of the Ca^{2+} dysregulation might help in the development of new treatments in order to reduce ischemic brain injury. An optimal concentration of Ca^{2+} does not lead to neurotoxicity in the ischemic neuron. Intracellular Ca^{2+} act as a trigger for acute neurotoxicity and this cause induction of long-lasting processes leading to necrotic and/or apoptotic post-ischemic delayed neuronal death or of compensatory, neuroprotective mechanisms has increased considerably. Moreover, routes of ischemic Ca^{2+} influx to neurons, involvement of intracellular Ca^{2+} stores and Ca^{2+} buffers, spatial and temporal relations between ischemia-induced increases in intracellular Ca^{2+} concentration and neurotoxicity will further increase our understanding about underlying mechanism and they can act as a target for the development of drugs. Here, in our article we are trying to provide a brief overview of various Ca^{2+} influx pathways involve in ischemic neuron and how ischemic neuron attempts to counterbalance this calcium overload.

Keywords Ischemia · Calcium · Neurotoxicity

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Introduction

Cerebral ischemia is a state, which arises due to insufficient blood flow to the brain for the fulfillment of metabolic demand [1]. Insufficient blood supply results in deprived oxygen supply or cerebral hypoxia leads to the death of brain tissue or cerebral infarction / ischemic stroke [2]. This may represent as sub-type of stroke along with subarachnoid hemorrhage and intracerebral hemorrhage [3]. Ischemia may results in an alteration in metabolism, reduced metabolic rate and causes the energy crisis.

In ischemic stroke, blood flow get interrupted, leads to the reduced oxygen supply. In a state of oxygen depletion and low availability of metabolic substrate with the brain, inability to use anaerobic respiration causes severe ATP deficiency. In absence of ATP ion channels, especially Na^{+}/K^{+} ATPase is unable to maintain normal electrochemical gradient, which leads to the sustained depolarization of glia and neuronal cell [4]. In sustained membrane depolarized cell, voltage-gated Ca^{2+} channels are open causes and insufficient Ca^{2+} pump activity along with ATP deficiency leads to increased intracellular Ca^{2+} concentration. Increased intracellular Ca^{2+} causes release of excitatory neurotransmitter especially, glutamate extracellularly. In the absence of ATP, reuptake of excitatory

glutamate get reduced making an excess of glutamate in extracellular space. Glutamate gets binds to the ionotropic receptors (iGluRs), especially NMDA (N-methyl- D-aspartic acid) and AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) present at the postsynaptic neurons and causes massive Ca^{2+} influx and consequent excitotoxicity. A continued Ca^{2+} influx causes activation of the phospholipases and proteases and also induces the generation of the free radical species (ROS). Activation of the phospholipases causes cell membrane disintegration, allow water to enter into the cell. Phospholipases degrade the cell membrane, allowing water to cross into the cell. In addition, glutamate-mediated overstimulation causes Na^{+} and Cl^{-} enter to enter within the cell [5]. Inward movement of water within the cell causes endo-osmosis leads to the cell swelling, shrinking of the extracellular space and cytotoxic edema resulting necrosis. Small molecules at various point to inhibit and to attenuate cerebral ischemic injury can, potentially target this ischemic cascade.

The resultant energy crisis leads to the activation of a cascade of counterproductive biochemical and physiological events leading to the acute or delayed death of a neuronal cell. Transient ATP synthesis inhibition leads to the delayed cell death and prolonged inhibition of ATP synthesis causes acute neuronal cell death [6]. Acute cell death is a condition where excessive intracellular calcium ion activates various calcium-binding proteins, which are phospholipases, endonucleases, and calpains that causes breakdown of a cell membrane and organelles resulting in necrosis-like cell death. Regulating calcium can affect several key molecules that may lead to neuronal cell death [7]. Here, we present several channels and protein that may regulate the calcium entry within the neurons, maintain homeostasis of the calcium within the neurons and identifying those, which are responsible for cell death, and its downstream signaling pathways may lead to improved strategies for treating ischemic and excitotoxic disorders [8].

Types of Ischemia Involved in Neuronal Cell Death

Regulation of calcium within the brain depends upon the type of neuronal ischemia. Here, we introduce the type of ischemia-causing neuronal cell death by regulating calcium homeostasis. Neuronal ischemia is the consequence of a stroke (cerebrovascular accident), comprise of condition with the reduced delivery of nutrients to brain neurons. Type of ischemia determines the extent of degeneration within the brain, which explain the extent of calcium ion progression within the brain.

Global or Forebrain Ischemia

A typical feature of this type of ischemia is a brief or intermediate duration, thus allowing recirculation and long-term recovery. The damage is often conspicuously delayed. Global ischemia, as occurs in cardiac arrest, can be sustained only for periods of up to 12 min exacting resuscitative measures are then required. For these reasons, a majority of researchers now use forebrain ischemia caused by carotid artery clamping in gerbils and rats, either alone (gerbils) or combined with vertebral artery occlusion or hypotension (rats) [9].

Focal Ischemia

Focal ischemia is caused by occlusion of a middle cerebral artery. The ischemia is usually less severe and for a longer duration. One can distinguish between a core of tissue (the focus) with relatively dense ischemia and perifocal tissues (penumbra), which are less densely ischemic because they receive a collateral blood supply from leptomeningeal branches of other major arteries. Cells in the focus are so poorly supplied with oxygen that they are doomed unless reperfusion can be quickly reinstituted, whereas those in the penumbra are at risk [10]. Both global and focal ischemia involve calcium hyperpolarization but at a smaller extent and generally results in the apoptosis of neurons.

Receptor, Channel and Other Cellular Component Leads to Calcium Entry Following Ischemia (Fig. 1)

Glutamate Receptors

Glutamate receptors are located on the cytoplasmic membrane of neurons and are activated following the binding of the neurotransmitter glutamate. Their main function following glutamate binding is to cause postsynaptic excitatory transmission. Glutamate receptors can be divided into two broad groups based on selective affinity for different agonists: (1) Ionotropic glutamate receptors which include N-methyl-D-aspartic acid (NMDA) receptors, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and kainic acid (KA) receptors; and (2) the metabotropic glutamate receptors (mGluR), which are activated selectively by quisqualate (an agonist of mGluR) [11, 12]. G-protein activation of phospholipase C is mediated by the metabotropic receptor, which interacts with other receptors on the cell membrane and converts phosphatidylinositol 6,7 bis-phosphate (PIP2) in the cell membrane to inositol tris-phosphate (IP3) and di-

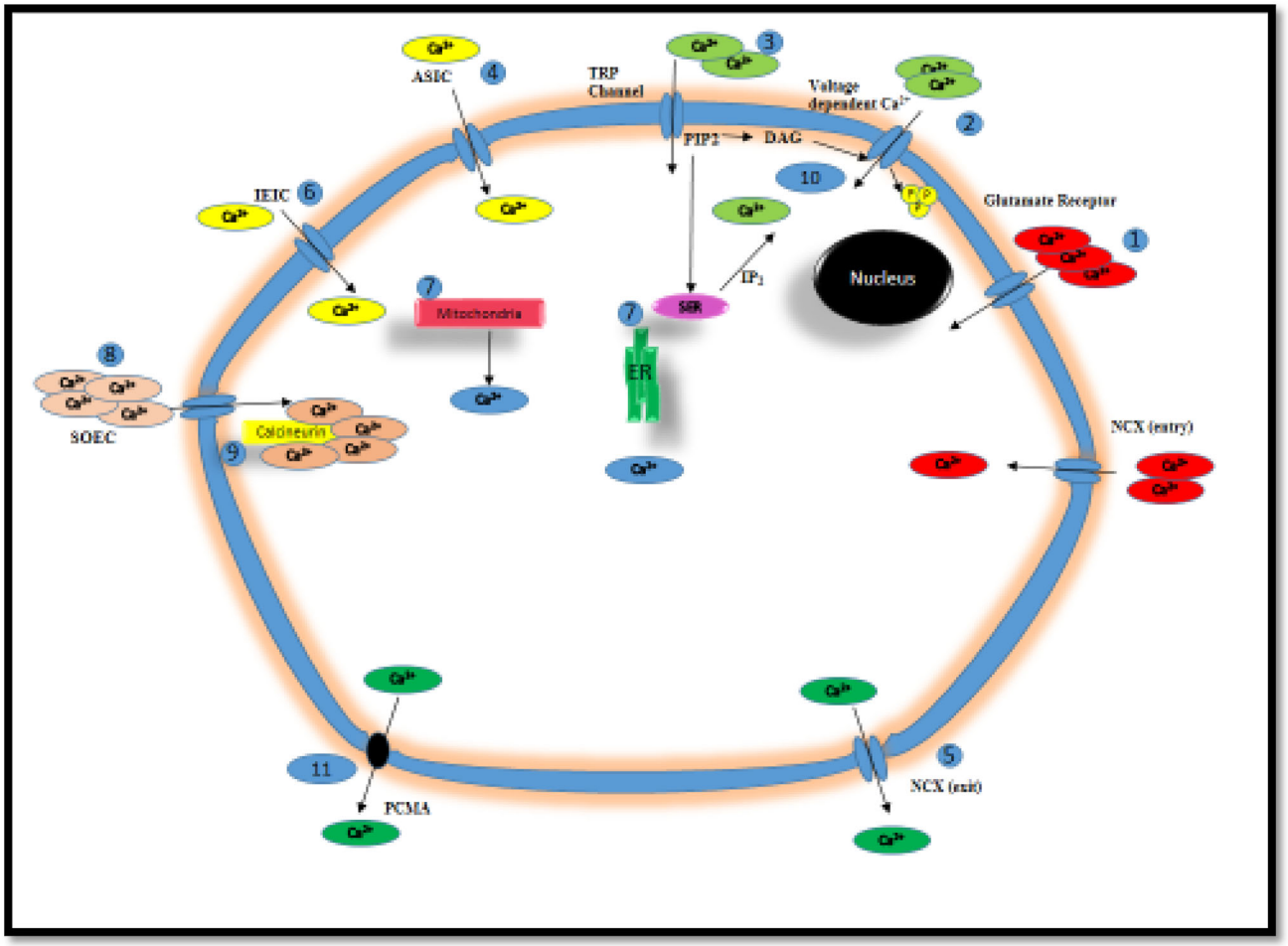

Fig. 1 Calcium entry and exit into neurons following cerebral ischemia. 1 Calcium entry via, 1.glutamate receptors (NMDA, AMPA, KA, and mGluR, 2. VDCCs (voltage-dependent calcium channel), 3.TRP (transient receptor potential channels), 4. ASIC (acid-sensing ion channels), 5. NCX (sodium-calcium exchanger operating in entry mode), 6. IEIC (inward excitotoxic injury current calcium permeable channels), 7. Calcium can also be sequestered intracellularly (middle of cell diagram) by the mitochondria and ER (endoplasmic reticulum), 8.SOCE (store-operated intracellular calcium entry), 9. Intracellular Calcium binding protein i.e. Calcineurin, 10. Calcium mediated intracellular signaling and Calcium exit via, 11. PCMA (Calcium ATPase pump) and 12. NCX (sodium-calcium exchanger operating in exit mode)

acylglycerol (DAG) [13]. IP3 acts to release calcium from the ER, whilst DAG activates protein kinase C (PKC) that mediates many effects. The release of calcium ions is necessary for activation of calcium-dependent PKC isoforms; similarly, PKC can phosphorylate proteins that can then change calcium signaling. With respect to ionotropic glutamate receptors during ischemia, excessive activation of NMDA and AMPA glutamate receptors is a major source of calcium influx [13]. Similarly, activation of definite metabotropic glutamate receptor subtypes can result in the release of calcium from the ER [14].

Ionotropic Glutamate Receptors

Also called as ligand-gated ion channel. Ionotropic receptors are membrane-bound receptor proteins that respond to ligand (glutamate) binding by opening an ion channel and allowing ions to flow within the cell, either increasing or decreasing the likelihood that an action potential will fire.

NMDA Receptors The NMDA receptor is a nonspecific cation channel having a high affinity for calcium ions. Extracellular glutamate causes activation of NMDA receptor leads to neuronal membrane depolarization and VDCCs-mediated calcium influx, in addition, calcium influx through the channel itself. NMDA receptors which contain the NR2A subunits have been shown to be located primarily in the synapse, whilst those receptors containing the NR2B subunits are located predominantly in the extrasynaptic zones of neurons [14].

Synaptic NMDA receptor activation Current evidence has present that activation of synaptic NMDA receptors is

related to a prosurvival response in neurons. It has been characterized by cultured neurons that, pro-survival response, is induced by a mild non-damaging level of NMDA receptor activation. The prosurvival response is associated with the up-regulation of BCL6, BTG2 i.e. prosurvival proteins and downregulation of CASP8AP2, DIDO1 i.e. pro-death proteins [15].

Extra-synaptic NMDA receptor activation In contrast to synaptic NMDA activation, overstimulation of extrasynaptic receptors triggers a neuronal damaging signaling response. For example, stimulation of extrasynaptic NMDA receptors can mediate upregulation of the CLCA1 (calcium-activated chloride channel) and activation of p38 (mitogen-activated protein kinase p38) both of which contribute to neuronal death [15–17]. In addition to NMDA receptor subcellular location, receptor subunit composition is also crucial in the characterization of the neuronal fate following cerebral ischemia [18].

AMPA Receptors AMPA receptors are nonspecific cation channels that consist of four subunits (GluR1-4), with receptor permeability to calcium-dependent on the configuration of the subunits. GluR2 subunit of AMPA assembly are impermeable to calcium, however, GluR1, GluR3 and GluR4 subunits are permeable to calcium ions [17].

Kainic Acid Receptors KA receptors are comprised of four subunits, containing a combination of one or more of five different subunits (KA1, KA2, and GluR5-7). Receptor subunit composition determines receptor permeability and function. They are permeable to sodium and potassium ions and generally not permeable for calcium ion [18]. Their role in neuronal fate following ischemia is not well understood, but there is evidence that their activation can stimulate survival pathways through regulation of the inhibitory neurotransmitter, γ-aminobutyric acid (GABA). For example, it is regarded that the post-synaptic KA receptor-mediated liberation of GABA triggers GABA receptors, leads to inhibition of ischemia-induced NMDA over-activation [18].

Metabotropic Glutamate Receptors

Metabotropic glutamate receptors can be divided into three different families with subtypes for each group consisting of Group I (mGluR1, mGluR5), Group II (mGluR2, mGluR3), and Group III (mGluR4, mGluR6-8). Metabotropic receptor-mediated causes release of calcium from the ER, these metabotropic receptors can give rise to increased intracellular calcium following ischemia. Moreover, it has also been demonstrated that metabotropic glutamate receptor agonists can be protective following ischemia [19].

Voltage-Dependent Calcium Channels

Voltage-dependent calcium channels (VDCCs) are a type of transmembrane ion channel found in excitable cells and are composed of four homologous α1 transmembrane subunits which form a calcium-permeable pore along with α2δ, β1−4, and γ auxiliary subunits which function in modulating the channel complex. There exist several structurally related subtypes, including L-type, N-type, P/Q-type and T-type. The ischemic event involves, neuronal membrane depolarization leads to the activation of these VDCCs channels and intracellular calcium influx [20].

L-Type VDCCs

L-type VDCCs (otherwise known as long-lasting or DHP receptors) are commonly found on dendritic neurons and, when activated, trigger calcium influx and the expression of genes leading to cell survival. In early phases of ischemia and reperfusion, activation of the L-type channel is likely to contribute calcium dysregulation and cell death. Interestingly, in the later stages after ischemia/reperfusion L-type channels are down-regulated, a process that is thought to contribute to delayed neuronal death, as the administration of channel agonists in late post-ischemia settings is neuroprotective [21].

N-Type VDCCs

N-type VDCCs (otherwise known as neural) play a primary role in neurotransmitter release from the presynaptic terminal via the influx of calcium after depolarization. The toxin ω′ -conotoxin is a specific blocker of these channels and is regularly used to study their function and mechanisms. Early studies revealed that a synthetic peptide, SNX-111 (a selective N-type VDCCs blocker), was found to be highly neuroprotective following global cerebral ischemia, suggesting that N-type calcium channels play an important role in calcium associated ischemia and neuronal injury [22].

P/Q-Type VDCCs

P/Q-type VDCCs (or Purkinje) are found mainly in the cerebellum and are involved in presynaptic neurotransmitter release. ω′ -agatoxin used as a blocker for the reducing brain infarcts following focal cerebral ischemia [23].

T-Type VDCCs

Transient-type VDCCs imparted low-voltage activity within the brain and its activation occurs at resting phase of the neurons (~ -60 mV) accessing small amounts of calcium influx, which is very crucial for signal amplification. Inhibitors specific to these channels have been shown to dramatically reduce neuronal damage in hippocampal slice cultures following oxygen-glucose deprivation [24].

Transient Receptor Potential Channels

Transient receptor potential (TRP) channels are a family of nonselective cation channels for ions such as magnesium, sodium, and calcium. TRP channels consist of six transmembrane segments with pore formation between segments 5 and 6. These TRP channels in mammals can be categorized into six subfamilies: TRPC (canonical), TRPP (polycystin), TRPA (ankyrin), TRPV (vanilloid), TRPML (mucolipin), TRPM (melastatin), and TRPA (ankyrin). Down-regulation of TRPM7 is neuroprotective following global ischemia. Stored calcium depletion leads to opening of the TRPC channels, and the ensuing calcium entry provides the calcium necessary for complete refilling of the calcium store. Therefore from the above-mentioned literature, it has been clear that these receptors are likely to contribute to neuronal intracellular calcium influx following ischemia [25].

Acid-Sensing Ion Channels

Acid sensing ion channels (ASICs) are the nonselective ion channel, activated in response to the decreased extracellular pH. An episode of cerebral ischemia is comprised of the decreased extracellular pH leads to activation and opening of the ASIC channel this makes calcium to enter within the neurons. Moreover, it has been manifested that NR2B-containing NMDA receptors can trigger the calcium/calmodulin-dependent protein kinase II (CaMKII) pathway leads to phosphorylation of the ASIC1a channel resulting in acid toxicity-induced cell death [26].

Sodium-Calcium Exchanger

Sodium-calcium exchanger (NCX) structure consists of nine trans-membrane segments, which are involved in binding and transportation of sodium and calcium ions and a large intracellular hydrophilic loop which functions to regulate NCX activity. This arises as conflicting opinions as to whether NCX is neuroprotective or neuroimaging. Under these conditions-blocking NCX activity is neuroprotective [27]. In contrast, after milder episodes of cerebral ischemia, which normally results in neuronal recovery of delayed neuronal death, the NCX operates in calcium exit mode in an attempt to restore calcium homeostasis. At this point, proteolytic inactivation of NCX3 has been elaborated which occur following cerebral ischemia, rendering the channel inactive and resulting in reduced calcium efflux, contributing to calcium dysregulation and cell death [28].

Calcium- Permeable Channel

In a recent study, a novel calcium-permeable channel is found in cultured hippocampal neurons which is acts as inward excitotoxic injury channel (IEIC), which the authors believe is also responsible for glutamate-induced extended neuronal depolarization (END) and calcium-mediated excitotoxicity. Depending on in vitro experimental studies, it is found that IEIC is activated after an excitotoxic insult and once activated results in sustained neuronal calcium entry. Moreover, later investigations display that blocking of the IEIC by gadolinium following excitotoxicity attenuated sustained calcium influx and prevented neuronal death [29].

Intracellular Calcium Sequestering and Release: Release from Mitochondria and Endoplasmic Reticulum

Mitochondria

Mitochondria mainly function to produce energy intermediates as, NADH and ATP via tri-carboxylic acid cycle and oxidative phosphorylation. In excitable cells such as neurons, mitochondria play a role in regulating intracellular calcium levels. Mitochondrial calcium concentration can be maintained either by the exchange of calcium ions through the matrix for cytosolic sodium ions mediated by mitochondrial sodium/calcium exchanger (NCXMITO), located at the inner mitochondrial membrane [30].

Endoplasmic Reticulum

Endoplasmic reticulum (ER) act as a storage house for calcium in neurons and other cells. ER take part in the fundamental homeostatic role during and following cerebral ischemia, by sequestering excess cytosolic calcium, which is thought to prevent ER stress and thus provide a protective mechanism against cell death. Normally, ER calcium influx is controlled by the Ca^{2+}-ATPase pump located on the ER membrane, but during and following ischemia, its function in neurons is compromised due to declining ATP levels. Activation of P3Rs by IP3 results in a rapid release of calcium from the ER [31].

Store-Operated Intracellular Calcium Entry

In store-operated calcium entry (SOICE) or capacitive calcium entry influx of extracellular calcium occur across the plasma membrane from store-operated calcium channels, in a condition of ER intracellular calcium release and store depletion. Recently, this calcium entry mechanism has been demonstrated to occur following cerebral ischemia and contribute to neuronal death. Activated store-operated channels, is regulated with the help of ER transmembrane sensing protein STIM2 (stromal interaction molecule). STIM2 when activated gets bind with and stimulate store-operated calcium channels [32].

Cyclic adenosine monophosphate

Cyclic adenosine monophosphate (cAMP) has been seen in neurons that various ion channel is regulated by means of dependent protein kinases including neuronal. It has been observed that in the cardiac tissue a stimulatory effect of the catecholamines on calcium currents is transmitted by beta-adrenergic receptors related with adenylate cyclase activation. This process is duplicated by administration of cAMP or by the catalytic subunit of cAMP-dependent protein kinase indicating that regulation of Ca^{2+} channels by cAMP is through protein phosphorylation [33].

Phosphatidylinositol Turnover

Phosphatidylinositol turnover is also regulated to the calcium level in the neurons. Many of the research study documented that stimuli initiated by the receptor have relation with the phosphatidylinositol turnover. It done studies documented that receptor linked phosphodiesterase cause's liberation of the inositol 1, 4, 5-trisphosphate and diacylglycerol from the plasma membrane phospholipid phosphatidyl 4, 5-bisphosphate. Subsequently, inositol 1, 4, 5-trisphosphate will mobilize intracellular calcium, as it gets bind to the Ca^{2+}-ATPase channel present on the ER membrane, thereby stimulating it to release calcium from ER to the cytoplasm. Despite that, it may appear as the potential pathway that may directly affect plasma membranes by increasing permeability to extracellular Ca^{2+} and loss of membrane-bound calcium [34].

Protein Kinase C

Protein Kinase C, which promotes calcium current via phosphorylation of ion channels. Breakdown of phosphatidylinositol 4,5-bisphosphate into di-acylglycerol activates protein kinase C, this may promote calcium currents by phosphorylation of ion channels. In hippocampal neuron protein kinase C implicated in modulating ionic conductance [35]. Acetylcholine, histamine, serotonin, substance P, vasopressin, and cholecystokinin are the neurotransmitters that utilize this protein kinase C pathway [36].

Phospholipid Methylation

Methylation of phosphatidylethanolamine to phosphatidylcholine promotes calcium influx. Phospholipid methylation may be important as it does occur with stimulation of benzodiazepine receptors and during hormonal manipulation of astrocytoma cultures [37].

Other Ca^{2+} Regulators

Majority of the intracellular calcium is sequestered or bound by the endoplasmic reticulum, mitochondria, and calcium binding proteins. Calmodulin binds with free calcium although there may be other distinct Ca^{2+} binding proteins within neurons. Some evidence which indicates that calcium is bound to glycoproteins present at the outer plasma membrane and to phospholipids along the inner aspect [38].

Calcium Homeostatic Mechanism in Ischemic Neuron

Calcium ATP-ase Pump

The calcium ATPase pump, for example, plasma membrane Calcium ATPase pump i.e. PMCA involve in regulation of the intracellular calcium through active expelling of calcium out of the cell. Its structure consists of ten transmembrane domains which form the calcium-permeable pore and three intracellular loops which regulate its activity. Moreover, ischemia causes activation of the caspase that might cleave and thereby inactive PMCA in neurons resulting in the calcium overload [39].

Sodium Calcium Exchanger (NCX)/Calcium Exit Mode)

Under normal physiological conditions, NCX acts as a calcium extrusion transporter by operating in the forward or calcium exit mode [40]. The beneficial effects of NCX activity following cerebral ischemia are further supported by data showing that NCX knockout mice suffer more brain injury following both global and focal cerebral ischemia [41].

Ca^{2+} Channel Inhibitors

Ca^{2+} is the key component involved in the progression of ischemia, and its inhibition is directly correlated with the ischemic neuroprotection [42]. Magnesium is generally considered as natural calcium blocker as it competes with Ca^{2+} for Ca^{2+} receptor binding or passage through the ion channel [43]. Nimodipine is a Ca^{2+} channel inhibitor, which can pass the BBB and dilates cerebral blood vessels [44]. Phase III clinical trials (VENUS) had been done for evaluation of Nimodipine for ischemic stroke treatment [45]. Nymalize, an oral Nimodipine derivative, FDA approved for the treatment of patients with specific cerebral hemorrhage [46, 47].

Conclusion

Calcium plays a crucial role in during ischemic state of neurons. This review provides a demonstration of a various pathway, which imparts in the calcium hyperpolarization. These calcium signaling receptors and channels act as a possible drug target for the suppression of calcium signaling occur during ischemia.

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Compliance with Ethical Standards

Conflict of interests The authors declare that they have no conflict of interests.

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