Ion channels in neuronal survival

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The study of ion channels represents one of the most active fields in neuroscience research in China. In the last 10 years, active research in various Chinese neuroscience institutions has sought to understand the mechanisms responsible for sensory processing, neural development and neurogenesis, neural plasticity, as well as pathogenesis. In addition, extensive studies have been directed to measure ion channel activity, structure-function relationships, as well as many other biophysical and biochemical properties. This review focuses on the progress achieved in the investigation of ion channels in neuronal survival during the past 10 years in China.

ion channels, neuronal survival and ischemia.


Neuronal activity relies on rapid changes in the electrical potential difference across the cell membrane. These rapid changes in membrane potential are mediated by ion channels, a family of membrane proteins found in cells. As fundamental determinants of neuronal ion homeostasis, ion channels are essential for a variety of neuronal functions, including neuronal survival. Among the families of ion channels, the channels that regulate intracellular Ca\(^{2+}\) concentration are critical factors in controlling neuronal survival. It has been known that Ca\(^{2+}\) influx via the voltage-dependent Ca\(^{2+}\) channels (VDCCs) promotes neuronal survival through a mitogen-activated protein kinase (MAPK) and cAMP-response element binding protein (CREB)-dependent mechanism [1]. Therefore, increases in VGCC activity or its expression level lead to neuroprotection. By contrast, abnormal ion channel function or expression results in neuronal damage. For example, over-stimulation of the NMDA-subtype of glutamate receptors in ischemia leads to the overload of Ca\(^{2+}\) and consequent excitotoxicity in neurons [2]. The Ca\(^{2+}\) influx through the acid-sensing ion channels (ASICs) is thought to be responsible for neuronal damage in ischemia. Therefore, it appears that Ca\(^{2+}\) influx through different channels leads to different outcomes in neuronal survival. Because of their widespread functional significance, cell surface location, structural heterogeneity and restricted tissue expression, ion channels are also attractive targets for drug development. In this review, we will discuss the idea that the Ca\(^{2+}\) influx through different channels leads to the opposite outcome in neuronal survival. We will summarize the findings about the role of ASICs and kainate-type glutamate receptors in neuronal damage and the role of VDCCs and transient receptor potential canonical (TRPC) channels in neuronal survival.

1 Acid-sensing ion channels (ASICs)

ASICs are activated by extracellular protons [3–5] and belong to the degenerin/epithelial Na\(^{+}\) channel superfamily. These channels are cation selective and sensitive to diuretic amiloride [6,7]. To date, 4 genes have been cloned: 1, 2, 3 and 4, encoding 6 subunits of ASICs (1a, 1b, 2a, 2b, 3, and...
ASIC1a homomultimers conduct Ca\(^{2+}\) [7,9,10,14], blocking ASIC1a may have a longer therapeutic time window in preventing ischemic cell death, therefore, it is a potential therapeutic target for stroke. In contrast to the limits of non-NMDARs [15] and ASICs [7], ischemia-induced neuronal death may not be mediated by NMDARs and AMPARs [29,30]. Those findings by Zhang and his colleagues have established a protective function in this process. This data suggests that GluR6 mediates JNK activation resulting in neuronal cell death via nuclear and non-nuclear pathways. In addition, they recently found that GluR6-containing KARs mediated p38 MAP kinase activation, and then induced increased phosphorylation of MAPKAPK-2 during ischemia injury, and ultimately resulted in neuronal cell death in the rat hippocampal CA1 region [28].

It is well known that NMDARs and AMPARs play important roles in ischemia-induced cell death. JNK activation-induced neuronal death may not be mediated by NMDARs and AMPARs [29,30]. Those findings by Zhang and his colleagues suggest that, at least in mediating JNK activation and subsequent cell death, KARs are much more crucial than NMDARs and AMPARs.

In addition, Zhang and his colleagues [31] showed that a selective GluR5 agonist had a neuroprotective effect against ischemia-induced neuronal cell death in vivo. They found that GluR5-containing KARs activation suppressed Src tyrosine phosphorylation and decreased NMDAR activation through attenuating tyrosine phosphorylation of two NMDAR subunits: NR2A and NR2B. Thus, in contrast to GluR6-containing KARs, GluR5-containing KARs showed a neuroprotective effect against ischemia-induced neuronal cell death.

The findings obtained by Zhang and his colleagues have provided strong evidence for the involvement of KARs during cerebral ischemia, and suggest new potential ap-
proaches for stroke therapy other than blocking NMDARs.

3 Voltage-gated Ca\(^{2+}\) channels

Acute ischemic stroke produces an infarct core, in which the cell death is rapid and irreversible, and a peri-infarct area, in which the cell death is delayed and rescuable. Transient forebrain ischemia also induces delayed, selective neuronal death in the CA1 region of the hippocampus. The expression of a group of genes required for neuronal survival is regulated by L-type voltage-gated Ca\(^{2+}\) channels. Ca\(^{2+}\)-overloading as a result of over-stimulation of NMDA receptors is detrimental for neuronal survival [32]. Therefore, Ca\(^{2+}\) influx through different routes could lead to different outcomes in neuronal survival. Because rescuing neurons from ischemic brain damage by blocking the NMDA-subtype of glutamate receptors, known as a vital factor for Ca\(^{2+}\)-overloading in ischemia, has been unsuccessful, enhancement of neuronal survival might be an alternative measure to reduce brain damage in ischemia. Gao and colleagues [33] at Southern Medical University have examined by patch-clamp techniques whether changes in L-type voltage-gated Ca\(^{2+}\) channel activity in the CA1 and CA3 pyramidal neurons of the rat hippocampus after transient forebrain ischemia are involved in neuronal survival in ischemia. They found that in CA1 neurons vulnerable to ischemic insults, L-type voltage-gated Ca\(^{2+}\)-channel activity was persistently inhibited after ischemic insult, whereas in CA3 neurons invulnerable to ischemic insults, its activity was unaffected. They found that the possible oxidation of post-ischemic channel proteins was responsible for the down-regulation of the L-type calcium channel activity. Inhibition of the L-type, but neither the N-type nor the P/Q-type Ca\(^{2+}\)-channels, markedly suppressed the cultured hippocampal neuron survival. By contrast, a specific L-type voltage-gated Ca\(^{2+}\)-channel agonist reduced neuronal cell death and restored the channel activity suppressed by the nitric oxide donor. These results suggest that the L-type Ca\(^{2+}\)-channel activity, a prosurvival activity, was specifically inhibited in ischemia.

The L-type voltage-gated Ca\(^{2+}\)-channel agonist applied after reoxygenation or reperfusion greatly decreased neuronal injury in the oxygen-glucose deprivation, a condition used as an in vitro ischemic model. Similarly, activation of L-type Ca\(^{2+}\)-channels led to neuron protection in animals subjected to forebrain ischemia-reperfusion. These results suggest that ischemia-induced inhibition of L-type Ca\(^{2+}\) currents may be responsible for the delayed death of neurons in the CA1 region, possibly via oxidation mechanisms. These findings have at least two major implications: First, preservation of neuronal survival might be a new perspective on prevention of neuronal death in ischemia; Second, L-type voltage-gated Ca\(^{2+}\)-channels might serve as a novel target for neuroprotection at late stages of reperfusion after ischemia.

Gao and colleagues also showed that the expression levels of the L-type Ca\(^{2+}\)-channel proteins were not altered in ischemia. Therefore, they speculated that functional down-regulation was likely due to changes in oxidation state of the channel proteins and was critical for neuronal damage in ischemia. How L-type Ca\(^{2+}\)-channel activity was down-regulated in the late stage of reperfusion remains unclear. The relationship between the Ca\(^{2+}\) influx in the early stage of the reperfusion and that in the later stage; and whether blocking the Ca\(^{2+}\) influx in the early stage of the reperfusion could affect that in the later stage remain unknown. Elucidation of these questions will enhance our understanding on the mechanism by which Ca\(^{2+}\) influx affects neuronal survival in the later stage of reperfusion.

4 Transient receptor potential canonical (TRPC) channels

The formation of a neuron network is one of the most significant consequences of brain development, in which neurons are connected through the synapse, a functional and structural unit found between axons and dendrites. The establishment of the network requires complicated and regulated processes, including neuron survival, formation of polarity, axonal pathfinding and synaptogenesis. Ca\(^{2+}\) is necessary for neuronal survival during development. Wang and colleagues [34] at the Institute of Neuroscience of CAS in Shanghai found that the transient receptor potential canonical (TRPC) channel plays a critical role in granular cell survival during cerebellum development.

The TRP protein was first found in the mutant retina of Drosophila, in which the fly photoreceptor response to a continued light stimulation exhibited a transient depolarization. The TRP superfamily contains at least seven subfamilies based on sequence homolog, including TRPA, TRPC, TRPM, TRPN, TRPP, TRPV and TRPML. The mammalian TRPC proteins are first identified as homologs of Drosophila TRPs [35,36]. TRPC channels permeable to Ca\(^{2+}\) are formed by homomeric or heteromeric complexes of the TRPC proteins that constitute seven subunits [37]: TRPC 1-7 with an exception for TRPC2, a pseudogene in humans. These channels are non-selective cation channels permeable to Ca\(^{2+}\). The activation mechanisms of TRPCs involve stimulation of phospholipase C (PLC) by either receptor tyrosine-kinase or G-protein coupled receptors. As a result of the activation of PLC, both DAG and IP\(_3\) are generated through the hydrolysis of PIP\(_2\) and DAG or IP\(_3\) activates TRPC channels. Wang and colleagues [34] asked whether TRPC channels are involved in neuronal survival in the rat brain and cerebellum granule neuron (CGN) cultures. They found that down-regulation of TRPC3/6 in the rat cerebellum by in vivo electroporation of the specific RNAi against TRPC3/6 increased CGN apoptosis and this effect was res-
cued by over-expressing TRPC3 or TRPC6 in the cerebellum. In CGN cultures, down-regulating TRPC3/6 expression or blocking their functions suppressed brain-derived neurotrophic factor (BDNF)-dependent neuronal protection against apoptosis induced by serum deprivation. Ca\(^{2+}\) influx through voltage-gated Ca\(^{2+}\) channels activates the MAPK pathway leading to phosphorylation and the activation of CREB. The activation of CREB triggers transcriptional responses resulting in the enhancement of expression of pro-survival factors, such as BDNF or Bcl-2. Wang and colleagues [34] further showed that TRPC3/6-mediated intracellular Ca\(^{2+}\) elevation induced by BDNF is required for the phosphorylation or activation of MAPK and CREB, a well-established mechanism for neuronal survival. Inhibition or down-regulation of the PLC/IP\(_3\) receptor, which is known to activate TRPC channels, suppressed BDNF-dependent protection of CGNs. They thus proposed that binding of BDNF to its receptor stimulates PLC to generate IP\(_3\), leading to activation of TRPC3/6. The activated TRPC3/6 mediate Ca\(^{2+}\) influx and stimulate both calmodulin-dependent protein kinase and MAPK pathways, which converge on the activation of CREB to promote neuronal survival. Their findings provide direct \textit{in vivo} and \textit{in vitro} evidence of the significance of TRPC channels in promoting neuronal survival.

In the developing central and peripheral nervous systems, neuronal survival may depend on neurotrophic factors released by target tissues [38]. For example, BDNF, a member of the neurotrophin family of growth factors, is essential for the survival of a variety of neurons, including cerebellar granule cells. Receptors for neurotrophic factors are often receptor tyrosine kinases, which stimulate PLC-\(\gamma\) to activate TRPC channels [39]. In pontine neurons, BDNF triggers a nonselective cationic conductance that resembles TRPC channels, which are permeant to Ca\(^{2+}\). As Ca\(^{2+}\) is known to mediate a variety of physiological functions, including cell growth and survival, it is possible that Ca\(^{2+}\) influx through TRPC channels is required for the neuronal protection effect of neurotrophic factors, such as BDNF. Thus, TRPC channels might act as potential cellular sensors for environmental cues, especially for the trophic factors. Future development of specific agonists or antagonists to these channels or generation of conditional knockout of these proteins enhance our understanding of the significance of these channels in neuron survival during brain development or under pathological conditions.

Taken together, the results summarized above are consistent with the explanation that Ca\(^{2+}\) influx via ASICs and kainate receptors induces neuronal damage, whereas that via L-type voltage-gated Ca\(^{2+}\) channels and TRPC3/6 leads to neuronal protection (Figure 1). The mechanisms underlying these paradoxical phenomena are not entirely clear at present. It is possible that the kinetics of Ca\(^{2+}\) influx via these channels is different and that the downstream molecules from these channels are different. These differences might be involved in their diverse functions in neuronal survival. As our understanding of the complexity of ion channels in neuronal protection increases, the research activities in China concerning the significance of ion channels in neuronal survival have also been diversified. We have reviewed a few examples of recent work in China. With substantial government funding and the devotion of Chinese scientists to the systematic investigation of ion channels, accelerated progress is likely to occur.

**Figure 1** Ion channels, ion homeostasis and neuronal survival. TRPC3/6 channels, GluR5-contained KA receptors, GABA\(_{\text{A}}\) receptors, voltage-dependent Ca\(^{2+}\) channels (VDCCs) and ATP-dependent K\(^+\) channels (K\(_{\text{v}}\)) are necessary for maintaining intracellular ion homeostasis and promoting neuronal survival. After neuronal injury, glutamate receptors (including NMDA receptors, AMPA receptors and Glur6-contained KA receptors) and ASICs are respectively activated by extracellular glutamate and H\(^+\). The activation of these channels induces intracellular Ca\(^{2+}\) imbalance, activates downstream signalling pathways and causes neuronal cell death.
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Research Interests

Apoptosis occurs during development and may contribute to numerous pathological conditions, including stroke, spinal cord injury and certain neurodegenerative diseases. Although the molecular mechanism of apoptosis has been extensively investigated, how apoptosis is induced in neurons under pathological conditions, such as stroke, remains largely unknown. Our group has been studying the mechanism of neuronal cell death at the molecular and cellular levels, focusing on the role of intracellular ions and their channels in neuronal apoptosis.

The activation of ion channels and the subsequent stimulation of protein kinase cascades affect neuronal survival. Our ongoing research includes study of the functions and mechanisms of transient receptor potential (TRP) C channels and K⁺ channels in neuronal survival, differentiation and cell proliferation. A variety of methods including molecular biology, cellular biology, biochemistry and electrophysiology are employed to investigate the mechanisms by which these channels affect cell survival in the models of primary neuronal cultures, cell lines and animals.

We are interested in ion channel structure, function and regulation. Ion channels, which underlie the generation of electrical signals and information processing in the central nervous system, are crucial for controlling neuronal excitability, synaptic function and cell survival. Because of their widespread functional importance, cell surface location, structural heterogeneity and restricted tissue expression, ion channels are attractive targets for drug therapy. Our current research primarily focuses on the acid-sensing ion channels (ASICs). ASICs are cation-selective channels activated by extracellular hydrogen ions (protons, H⁺) and are involved in sensory perception and integration. Our previous studies have suggested that ASICs are important mediators of ischemic cell death and chronic pain. These results suggest that antagonists of ASICs hold promises as therapeutic agents. In addition, we are studying the regulation and function of GABA(A) receptor- and glycine receptor-chloride channels. We hope that our current research can provide scientific evidence leading to new therapeutic strategies.