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Review Article

TRPM7 is a unique target for therapeutic intervention of stroke

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Abstract: Ischemic stroke is a leading cause of death and long-term disabilities. The current therapy is limited to thrombolysis and mechanical recanalization, which have limited success. A better understanding of the mechanisms underlying ischemic brain injury is therefore needed for the development of more effective interventions. Glutamate receptor-mediated Ca^{2+} overload and neurotoxicity have been well established for decades. However, clinical trials failed to show a satisfactory effect with the antagonists of glutamate receptors. Other glutamate-independent mechanisms, such as activation of acid-sensing ion channels and transient receptor potential melastatin 7 (TRPM7), have recently emerged as important events responsible for neuronal injury under ischemic conditions. In this review, we discuss how TRPM7 channels participate in ischemic brain injury.

Keywords: Stroke, neurotoxicity, TRPM7, Ca^{2+}, Zn^{2+}

Introduction

Ischemic stroke/brain ischemia is a leading cause of death and the most common reason for long-term disability. Current drug treatment is limited to thrombolysis using tPA. The success of tPA treatment is limited by multiple factors including the time lapsed for treatment and patient co-morbidity [1-3]. Pharmacological intervention to decrease the death of neurons has a potential to improve the patient outcome, either alone or combined with re-vasculization of obstructed artery. Revealing novel molecular mechanisms underlying ischemia-induced injuries will be essential to the design of new therapeutic interventions.

It has been recognized for several decades that over-activation of the glutamate receptors and subsequent Ca^{2+} toxicity plays a critical role in ischemic brain injury [4, 5]. Accordingly, antagonists of glutamate receptors have been shown to be effective in animal studies in protecting neurons against ischemic injury [4, 6, 7]. Unfortunately, clinical trials have failed to demonstrate a satisfactory effect by these agents in human [8-11]. Although multiple factors, such as severe side effects, have contributed to the failure of the trials, it is likely that blockade of glutamate receptors alone is not adequate to result in a significant improvement of ischemic outcome. In this regard, recent studies have provided strong evidence suggesting that glutamate-independent mechanisms, e.g., activation of acid-sensing ion channels (ASICs) or TRPM7 channels, also play an important role in ischemic brain injury [12-16]. In this short review, we focus on the role of TRPM7 channels in ischemic brain injury and its underlying mechanisms.

Structure of TRPM7

The transient receptor potential (TRP) is a superfamily of non-selective cation channels that are widely expressed in mammalian cells [17]. These channels play critical roles in the perception of a wide range of physical and chemical stimuli and in multiple fundamental cellular responses [17]. TRP channels have six putative transmembrane domains (TM), with intracellular N and C-termini. The pore region of TRP channels is formed by the loop between TM5 and TM6. There are seven subfamilies of TRP

channels: TRPC, TRPV, TRPM, TRPA, TRPN, TRPP and TRPML [17]. TRPM subfamily has eight members which includes TRPM7, a non-selective cation channel expressed in almost every tissue and cell type [18-20]. TRPM7 is also a chanzyme, with a kinase domain in its C-terminal region. A complete crystal structure for TRPM7 has not been resolved but the structure of a portion of the rat TRPM7 C-terminus has been reported [21]. It revealed a coiled-coil assembly domain critical for the formation of tetramers [21].

Electrophysiological characteristics of TRPM7 channels

Xiong and colleagues were the first to describe a cation conductance in neurons which can sense the change of divalent cations such as Ca^{2+} [22]. This was later on identified as mediated by TRPM7 [23]. In the presence of normal divalent cations, the permeability of TRPM7 channels to monovalent cations is decreased and the channels show outward rectification in whole-cell recordings with a reversal potential near 0 mV [20]. Upon removal or decrease of divalent cations, larger currents with an increased permeability to K^+ and Na^+ can be activated [22, 20]. As a non-selective cation channel, TRPM7 is highly permeable to divalent cations, with the following order of permeability: $Zn^{2+} \approx Ni^{2+} \geq Ba^{2+} > Co^{2+} > Mg^{2+} \geq Mn^{2+} \geq Sr^{2+} \geq Cd^{2+} \geq Ca^{2+}$ [24].

TRPM7 channels have unique features that make them a critical player for ischemic neuronal injury. On one hand, biochemical changes associated with brain ischemia facilitate the activation of TRPM7 channels. On the other hand, several electrophysiological characteristics of these channels likely make them more important than other targets for stroke intervention. (1) TRPM7 channel activity is enhanced upon the depletion of cellular ATP [25], a condition pertinent to brain ischemia. (2) TRPM7 current is potentiated by decreases of extracellular divalent cations [14, 19]. Following ischemia, influx of Ca^{2+} through voltage-gated calcium channels and NMDA receptors produces a decrease in the level of extracellular Ca^{2+} [26]. Although a reduction in the extracellular Ca^{2+} may decrease the driving force for Ca^{2+} entry, it causes a dramatic disinhibition of the TRPM7 channel, thus enhancing the overload of intracellular Ca^{2+}. (3) TRPM7 is potentiated by extracellular protons [27]. Following brain ischemia, marked reduction of tissue pH, a condition termed acidosis, occurs. Shortage of oxygen supply, for instance, enhances the anaerobic glucose metabolism, resulting in an accumulation of lactic acid [28, 29]. Energy shortage and ATP hydrolysis also releases H^+. In general, brain pH typically falls to ~6.5 [30, 31]. In severe ischemia or under diabetic condition, drops of pH to below 6.0 take place [30-32]. In contrast to its inhibitory effect on NMDA channels and voltage-gated calcium channels [33-35], acidic pH has been shown to enhance the TRPM7 current in HEK-293 cells, with up to 2-fold increase at pH 6.0 [27]. (4) TRPM7 is highly permeable to both Ca^{2+} and Zn^{2+} [24], two important players in ischemic neuronal injury. For several decades, Ca^{2+} toxicity is a well-recognized factor for ischemic brain injury [5]. Excessive Ca^{2+} influx and intracellular Ca^{2+} overload activates a cascade of cytotoxic events leading to inappropriate activation of several enzyme systems including the nitric oxide synthase (NOS), proteases, phospholipase A2 (PLA2) and the endonucleases. Overactivation of these enzymes in turn causes breakdown of proteins, lipids and nucleic acids [36-38]. Elevation of Ca^{2+} also causes neuronal damage by promoting the production of oxygen free radicals [39].

Similar to Ca^{2+} accumulation, intracellular accumulation of Zn^{2+} can also play an important role in neuronal injury after stroke [40, 41]. It has been demonstrated that the correlation between Zn^{2+} accumulation and cell viability is rather striking [40, 42-44]. (5) TRPM7 is activated by oxidative stress [14], a pathological condition pertinent to brain ischemia. The increased production of oxidants, such as NO and H_2O_2, activates or potentiates the action of TRPM7 channels [14, 44]. Furthermore, Ca^{2+} entry through TRPM7 may reinforce the production of reactive oxygen/nitrogen species, resulting in a further activation of TRPM7 and the development of a positive feedback loop that facilitates neuronal injury [45]. (6) Compared to other ion channels such as glutamate receptors and voltage-gated Ca^{2+} channels which show clear desensitization, TRPM7 channels conduct sustained currents that do not desensitize [23]. Taken together, these unique properties of TRPM7 channels likely make them a more important

player than glutamate receptors in ischemic brain injury.

Ca^{2+} toxicity mediated by TRPM7

The role of TRPM7 channels in ischemia-mediated neuronal injury has been well demonstrated both in *in vitro* and *in vivo* studies [14, 15]. In 2003, Aarts and colleagues were the first to demonstrate that treating cultured cortical neurons with prolonged oxygen-glucose deprivation produces an increase in Ca^{2+} influx and neuronal cell death. This Ca^{2+} influx and toxicity occur in the presence of the inhibitors of glutamate receptors and voltage-gated calcium channels [14]. The glutamate-independent Ca^{2+} toxicity can be however inhibited by non-specific inhibitors of TRPM7 channels and TRPM7 siRNA [14], providing strong *in vitro* evidence that TRPM7 channels are involved in ischemic neuronal injury. In 2009, Sun and colleagues provided *in vivo* evidence that TRPM7 knockdown protected the hippocampal CA1 neurons in a cardiac arrest model of brain ischemia [15]. As expected, TRPM7 knock down also attenuated ischemia-induced LTP impairment and preserved the memory related performance [15].

Zn^{2+} toxicity mediated by TRPM7

Despite convincing evidence that clearly demonstrated the role of Ca^{2+} toxicity in ischemic neuronal death, clinical trials targeting the Ca^{2+} entry pathways have had inconclusive results [9, 46]. Similar to Ca^{2+} toxicity, recent studies have suggested that zinc toxicity also plays an important role in neuronal injuries associated with various neurological conditions [41, 47]. The primary pathways mediating intracellular zinc accumulations and toxicity, however, remained unclear.

Some cation channels, e.g. voltage-dependent calcium channels and Ca^{2+}-permeable AMPA/kinate receptors, have been reported to show some zinc permeability [48, 49]. The activities of these channels may thus affect the intracellular zinc homeostasis and toxicity. Compared to the TRPM7 channels, these channels show desensitization and are more or less inhibited by acidic pH. These factors likely make their contribution to Zn^{2+} toxicity limited under ischemic conditions.

In addition to well-established Ca^{2+} permeability, TRPM7 is highly zinc permeable among the TRP family of ion channels [18, 24]. It is worth noting that the zinc permeability for TRPM7 channels is 4-fold higher than Ca^{2+} [24].

Despite these facts, there was no direct evidence to show that TRPM7 channels play a role in intracellular zinc dynamics at physiological/pathological relevant concentrations and more importantly, in zinc-mediated neurotoxicity. Using a combination of fluorescent zinc imaging, metal response element-based reporter gene assay, cell injury analysis and small interfering RNA techniques, Inoue and colleagues were the first to provide a strong evidence supporting that TRPM7 channels represent a novel pathway for intracellular zinc accumulation and zinc mediated neurotoxicity [50]. They showed that, in cultured mouse cortical neurons, addition of zinc at a concentration similar to that found in ischemic brain tissues produced significant neuronal injury. This Zn^{2+}-mediated neurotoxicity was reduced by non-specific TRPM7 channel blockers and by knockdown of the TRPM7 protein with siRNA. More relevant to brain ischemia, Zn^{2+}-mediated neuronal injury under OGD conditions was also diminished by TRPM7 knockdown [50]. In contrast, over-expression of TRPM7 in HEK-293 cells led to an increase in intracellular Zn^{2+} and subsequent Zn^{2+}-mediated cell injury [50]. Thus, Zn^{2+} entry through TRPM7 channels likely plays an important role in ischemic brain injury. Accordingly, agents that inhibit the activity of TRPM7 channels are expected to be protective against TRPM7-mediated Zn^{2+} toxicity. Indeed, local anesthetic lidocaine, which blocks TRPM7 channels, has been shown to attenuate TRPM7-mediated Zn^{2+} toxicity in neurons [51].

How does Zn^{2+} accumulation cause damage to neurons? Zn^{2+} accumulation likely contributes to catastrophic mitochondrial failure, loss of Ca^{2+} homeostasis and ROS release, resulting in acute necrosis. If a neuron survives an acute ischemic insult, other mechanisms may come into play [43]. For example, oxidative stress resulting from mitochondrial disruption, or NADPH-oxidase activation, can damage nuclear DNA, resulting in PARP activation. PARP activation results in PAR accumulation and NAD^+ depletion, which can result in metabolic/mito-

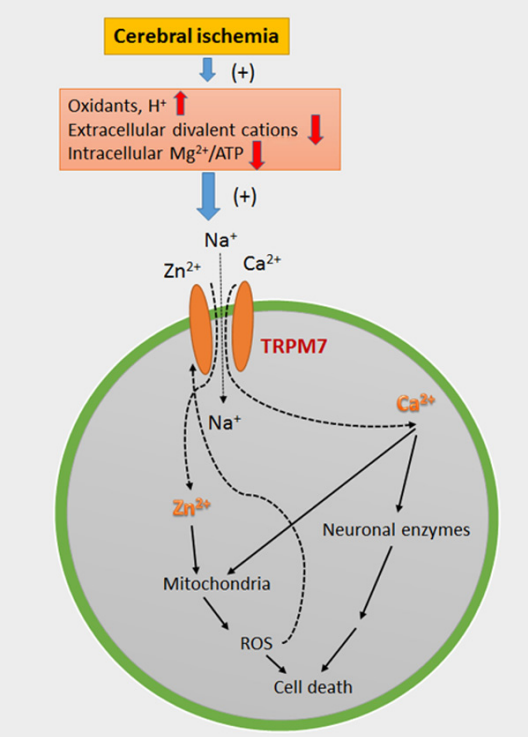

Figure 1. Biochemical changes following ischemia facilitate the activation of TRPM7 channels. Activation of TRPM7 channels induces accumulation of intracellular Ca^{2+} and Zn^{2+}, leading to neuronal cell death through different pathways.

chondrial inhibition. Consequent release of apoptotic mediators such as AIF and cytochrome C from mitochondria can lead to nuclear DNA cleavage and apoptosis, resulting in delayed neuronal injury. If a neuron is not killed by the above mechanisms, activation of P38 and/or ERK1/2 MAP kinases can contribute to slower apoptotic and non-apoptotic injury pathways [43].

Conclusion

Accumulating evidence suggest that activation of TRPM7 channels is a novel glutamate-independent mechanism involved in ischemic brain injury (**Figure 1**). Unlike other Ca^{2+} and Zn^{2+}-permeable channels which are, in general, inhibited by ischemic acidosis, TRPM7 channels have been shown to be potentiated by protons. In addition, TRPM7 conductance is sustained without desensitization. These properties likely make them more important than glutamate receptors in ischemic brain injury.

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