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·Review·

$P2X_7$ receptors in cerebral ischemia

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Cerebral ischemia is one of the most common diseases resulting in death and disability in aged people. It leads immediately to rapid energy failure, ATP depletion, and ionic imbalance, which increase extracellular ATP levels and accordingly activate $P2X_7$ receptors. These receptors are ATP-gated cation channels and widely distributed in nerve cells, especially in the immunocompetent cells of the brain. Currently, interest in the roles of $P2X_7$ receptors in ischemic brain injury is growing. In this review, we discuss recent research progress on the actions of $P2X_7$ receptors, their possible mechanisms in cerebral ischemia, and the potential therapeutic value of $P2X_7$ receptor antagonists which may provide a new target both for clinical and for research purposes.

Keywords: $P2X_7$ receptor; cerebral ischemia; neurotoxicity; calcium overload; neuroinflammation; neurotransmitter; receptor antagonist

Introduction

Cerebral ischemia remains a major cause of morbidity and mortality worldwide in aged people and affects millions every year. Immediately after ischemia, the cerebral blood flow dramatically decreases in the ischemic core, leading to rapid energy failure, ATP depletion, ionic imbalance, and neuronal death[1]. The cells in the penumbra (tissues surrounding the ischemic core), however, can retain their activity for a prolonged period. The neurodegenerative episodes and neuronal loss gradually expand from the ischemic core to the penumbra in the following several days. Therefore, effective interventions in this period are essential.

Extracellular ATP is an important signaling molecule mediating interactions among various types of cells in the central nervous system (CNS)[2]. It acts on cell-surface P2 purinoceptors and serves as a neurotransmitter, neuromodulator, and trophic factor under physiological conditions. On the other hand, pathological events such as mechanical or metabolic stress, inflammation, cellular injury, or changes in the ionic environment are all known to powerfully stimulate ATP release. This results in an ATP-rich extracellular environment leading to widespread activation of receptors on astrocytes and microglia and boosting diverse pathological cascades, such as glutamatergic excitotoxicity, and oxidative damage, as well as IL-1β and other cytokine-mediated signaling[3]. P2 receptors are classified into two subfamilies, P2X and P2Y. ATP couples to the ligand-gated P2X ion channel receptors and mediates fast excitatory neurotransmission, while ATP binds to the GTP-binding P2Y receptors to evoke slow excitatory responses. Seven distinct ionotropic P2X ($P2X_{1\text{-}7}$) and eight distinct metabotropic P2Y receptor subunits ($P2Y_{1,2,4,6,11\text{-}14}$), classified by electrophysiological properties and their amino-acid sequence homology, have been cloned from mammals[4].

P2X receptors are widely distributed in excitable and non-excitable cells of vertebrates. They play key roles in regulating afferent signaling (including pain), renal blood flow, the vascular endothelium, inflammatory responses, tumorigenesis, CNS injury, and embryonic development[5, 6]. Of the P2X family, the $P2X_7$ receptor ($P2X_7R$) has specific structural and pharmacological features and may play a prominent role in the progression of various neurological disorders, such as cerebral ischemic injury (stroke),

Alzheimer's disease, multiple sclerosis, amyotrophic lateral sclerosis, spinal cord injury, neuropathic pain and depression[3, 7, 8]. In the past few years, the potential role of this receptor in neuronal functions during cerebral ischemic stroke has received considerable attention.

Nevertheless, a major debate has emerged on the roles of activated $P2X_7$Rs in animal and cellular models of this disorder. Most indicate that up-regulation or over-activation of $P2X_7$Rs aggravate the brain tissue damage and sensorimotor deficit through several mechanisms such as Ca^{2+} overload which induces excitotoxic neuronal death or apoptosis, and receptor antagonists play a protective role[1, 4, 9-12]. However, a few reports are contradictory[13, 14]. In the following we thus review the unique properties of $P2X_7$Rs compared to other P2X receptor family members, the possible mechanisms of their actions in cerebral ischemia, and the potential therapeutic exploitation of $P2X_7$R antagonists.

$P2X_7$ Receptors and Their Unusual Properties Compared with Other P2X Family Receptors

The $P2X_7$R was first discovered by Buisman in 1988 and Gordon named it the P2z receptor[15] meaning "cell death receptor"[3] or "suicide receptor"[5] because its prolonged activation leads to cellular death. In 1996, the $P2X_7$R was first cloned from a rat brain cDNA library and classified as a member of the P2X receptor family[7].

$P2X_7$Rs have several properties that set them apart from other members of the P2X receptor family. First, the $P2X_7$R is 595 amino-acids long, having 35–40% homology with the other six members of the family[3]. Only the $P2X_7$R does not heterotrimerize with other P2X subunits. However, it was shown recently that $P2X_7$R subunits functionally interact with $P2X_4$ subunits in macrophages. The heteromeric $P2X_4$/$P2X_7$ receptor appears to have the pharmacological properties of both of the homomeric receptor subtypes[10]. Second, except for two transmembrane domains (M1 and M2) and a large extracellular loop containing the ATP-binding site[16], the $P2X_7$R has a unique structural feature: its intracellular carboxy terminal domain is much longer (239 amino-acids) than those of the other P2X receptor subunits (27–129 amino-acids). This long C-terminal tail can combine with many downstream proteins and lipids in the cytoplasm[7], and this is associated with unique functions as well as cellular localization[17]. Third, in contrast to other P2X receptors, the $P2X_7$R has a lower affinity for ATP[18], and only relatively higher concentrations of extracellular ATP (millimolar range) can activate $P2X_7$Rs relative to other P2X receptors (micromolar range)[7, 10, 19]. Last but not least, $P2X_7$Rs are bifunctional because they have two states, cation channels and plasma membrane pores[20]. When stimulated by lower concentrations of ATP, $P2X_7$Rs are mildly activated and function as small cation channels with a permeability significantly higher to Ca^{2+} than to Na^+ and K^+[9]. Repetitive or prolonged exposure to higher concentrations of ATP elicits progressive dilation of the receptor cation channel, which finally forms a large irreversible and nonselective transmembrane pore permeable to macromolecules up to 900 Da in size[1, 3, 21]. This promotes actin disaggregation, rapid cytoskeletal rearrangement, membrane blebbing, cytokine release, and finally apoptosis and cell death[9]. Wang *et al.*[22] reported that spinal cord injury is associated with high ATP release that lasts for at least 6 h after the initial injury. Exposure of $P2X_7$Rs on spinal cord neurons to ATP leads to high-frequency spiking, an irreversible increase in cytosolic Ca^{2+} and cell death, while $P2X_7$R antagonists inhibit this process and facilitate functional recovery. Another study indicated that the ATP concentration in the striatum of normoxic rats is 3.10 ± 0.34 nmol/L. During 220 min after middle cerebral artery occlusion (MCAO), extracellular ATP levels significantly increase to 5.90 ± 0.61 nmol/L[23]. In addition, stimulation of $P2X_7$Rs with high concentrations of ATP has dramatic cytotoxic actions in many neurodegenerative processes[24].

Activation of $P2X_7$ Receptors in Ischemic Brain Injury

$P2X_7$ Receptor Expression in the CNS under Physiological Conditions

Under normal conditions, $P2X_7$Rs are widespread on the membranes of neurons, astrocytes, microglia, oligodendrocytes, and oligodendrocyte precursor cells, as well as Schwann cells[14, 25-27], unlike the localization of $P2X_{1-6}$ receptor subtypes, which is mainly on the membranes of neurons[28]. They are especially abundant on microglia that are in a physiologically resting state,

display ramified morphology with numerous branching processes[20], and play protective roles such as host defense and tissue repair[28] against neuronal damage[29], suggesting that $P2X_7$Rs are involved in immune functions and inflammatory responses[13]. The cellular localization of $P2X_7$Rs in the CNS remains controversial. Yu *et al.* described the precise distribution of $P2X_7$ mRNA in the rat brain using isotopic *in situ* hybridization. Small $P2X_7$-positive glial-like cells are sporadically scattered in almost all areas of the brain[30]. In neurons, $P2X_7$Rs are primarily distributed on presynaptic terminals in the medulla oblongata and spinal cord, excitatory nerve terminals of CA1, CA3, the dentate gyrus and mossy fibers of the hippocampus, cortical synaptosomes, neuronal cell bodies of the medulla oblongata and hippocampus[1] and the nuclear envelope of postsynaptic neurons[11]. $P2X_7$Rs on the presynaptic membrane might serve as sensors of neuronal activity and extracellular ATP release, whereas the receptors on the nuclear envelope, regulated by cytoplasmic ATP, may be a mechanism of changing gene expression[31].

Altered Expression and Function of $P2X_7$ Receptors in Cerebral Ischemia

Many studies have suggested that, after cerebral ischemia, activated $P2X_7$Rs are expressed predominantly on activated microglia in the penumbra and on reactive microglia. Reactive microglia are in a state intermediate between activation and rest. They are at rest in shape but have positive $P2X_7$R immunoreactivity in remote projection areas such as the cingulate and medial frontal cortex and the synaptic terminals of neurons[4, 13]. Activation of $P2X_7$Rs contributes to post-anoxic depolarization and neuronal demise following ischemia[9].

Oxygen/glucose deprivation (OGD) in the *ex vivo* model of organotypic hippocampal slice cultures rapidly and transiently up-regulates $P2X_7$Rs in a time-dependent manner in the parallel fibers of the pyramidal cell layer in CA1 and CA2[11]. They directly participate in the metabolic impairment. OGD in mixed astrocytic/neuronal cultures selectively up-regulates $P2X_7$R immunoreactivity in the plasma membrane of neurons, but not astrocytes, 12 h after ischemia[25]. Post-ischemic, time-dependent up-regulation of the $P2X_7$R subtype on neurons and microglia in the MCAO model might be involved in neuronal apoptosis and tissue damage, suggesting a role of this receptor in the pathophysiology of cerebral ischemia *in vivo*[1, 4]. Besides, a transient and significant enhancement in $P2X_7$ protein expression was reported to occur immediately after asphyxia in a rat model of intrauterine asphyxia[32].

However, inconsistent with most reports that $P2X_7$R expression is increased in response to OGD *in vitro* or MCAO *in vivo*, Wang *et al.* showed decreased expression of $P2X_7$Rs in cultured oligodendrocyte precursor cells, the predominant oligodendrocyte-lineage stage in the cerebral hemispheres of the neonatal rat, after exposure to OGD for 2 h *in vitro*, and in a neonatal hypoxic-ischemic injury model in 3-day-old rats[14]. More recently, Zeng *et al.* reported that OGD decreases $P2X_7$R expression and modulates GSK-3β phosphorylation in radial glial clone L2.3 cells, and BzATP (a selective agonist of the $P2X_7$R) induces L2.3 cell death in a dose- and time-dependent manner[27]. However, Lee reported that in a rat model of anoxia-induced brain ischemia and reperfusion (I/R) injury, the whole brain $P2X_7$R expression is unchanged at 6 h after anoxia[33]. The differences in the maturation status of brain tissue, and the manner and distribution of hypoxic-ischemic damage between the perinatal and adult brain might explain these discrepancies. The $P2X_7$R-containing cells are lost in response to ischemia, which limits the detrimental consequences of post-ischemic brain injury. Depending on the diverse conditions of a given study, $P2X_7$Rs may increase, decrease, or even fail to alter the infarct size[1]. Further, polymorphisms of the $P2X_7$R gene might yield nonfunctional receptor protein and point mutations might cause trafficking defects or impaired function.

Although most studies demonstrated that blocking the $P2X_7$Rs improves the neuronal injury *in vivo* and *in vitro*[22, 34], Le Feuvre suggested that neither the knockout of $P2X_7$Rs nor $P2X_7$R antagonists affect the infarct volume in MCAO and in glutamate toxicity[12]. This may be explained by the activation of alternative compensatory pathways and thus suggests that the simple genetic deletion of $P2X_7$Rs might not be the right model to investigate their roles[3]. So far, little has been done to study $P2X_7$ knockout in ischemia, and further studies are needed to elucidate the exact effects and specific mechanisms underlying the $P2X_7$R knockout genotype in cerebral ischemia.

Possible Mechanisms of Action of Activated $P2X_7$ Receptors after Cerebral Ischemia

Activated $P2X_7$ Receptors Induce Ca^{2+} Overload Leading to Cell Death

Cerebral ischemia induces excessive ATP release into the extracellular space from the cytoplasm of damaged and dying cells due to nucleic acid degradation and membrane permeability[1, 35]. There are several possible modes of ATP release: (1) damaged neurons are depolarized by the elevated extracellular K^+ due to impairment of the Na^+-K^+ pump. ATP passively flows out along the electrochemical gradient through membrane channels of glial cells[23] or from the presynaptic vesicles exocytotically[35]; (2) organelles release ATP from astroglia in a Ca^{2+}-independent manner *via* $P2X_7R$ channels[25, 36]; and (3) $P2X_7Rs$ on activated amoeboid microglia and reactive microglia amplify the ATP signal and trigger ATP release through an autocrine process[37, 38]. In short, cerebral ischemia results in a sustained progressive elevation of ATP levels in the penumbra[22, 23].

The relatively high micromolar level of ATP in the extracellular space powerfully activates the $P2X_7Rs$ and leads to the formation of excitotoxic cytolytic pores[2, 39] and cell death.

Calcium overload is the prominent mechanism of $P2X_7R$-mediated excitotoxic cell injury in stroke as well as *in vitro* models. Activated $P2X_7Rs$ contribute directly to a rapid increase of intracellular Ca^{2+}[40] (due to the massive influx of extracellular Ca^{2+} *via* $P2X_7R$ membrane pores)[1, 41]. The resulting depolarization causes the secondary activation of voltage-gated Ca^{2+} channels[41-43], and intracellular Ca^{2+} continuously increases. The high concentration of intracellular Ca^{2+} activates nucleases and proteases (e.g., calpains) which eventually result in cell death[44]. Meanwhile, the mitochondrial matrix also takes Ca^{2+} from the cytoplasm. When the Ca^{2+} level in the mitochondrial matrix reaches a toxic threshold, the mitochondrial membrane potential collapses, the permeability transition pore opens, ions and solutes including Ca^{2+} flow into the cytosol[45] and activate the apoptotic caspase enzyme system[17]. Despite the massive downstream effects of Ca^{2+} overload, only a few intracellular signaling pathways have been described in detail. Currently, it has been shown that the ERK1/2 MAP kinase pathway contributes to the pathological consequences of OGD in the cultured hippocampal slice[43].

Pannexins are large membrane channels with broad expression throughout the body and have various functions. Pannexin-1 (Px1) is robustly expressed in the brain[47]. The opening of Px1 hemichannels is regulated by activated $P2X_7Rs$ under pathological conditions such as ischemia, and is responsible for the massive influx of Ca^{2+} from the extracellular space. Iwabuchi *et al.*[46] reported recently that in cultured astrocytes, the suppression of $P2X_7R$ activity during OGD results in the opening of Px1 hemichannels, leading to enhanced ATP release and astrocytic damage, while the released ATP further activates local $P2X_7Rs$, resulting in the closure of Px1 hemichannels, indicating the existence of a negative feedback loop. This negative feedback loop not only sustains astrocytic ionic homeostasis but also suppresses glutamate release *via* Px1 hemichannels.

Activated $P2X_7$ Receptors Induce Neuroinflammation as a Double-edged Sword

Numerous studies indicate that $P2X_7Rs$ also participate in the regulation of neuroinflammation and neuroimmune reactions as an inflammatory mediator in the CNS[49-53].

Microglia are the immune-competent cells in the CNS that participate in innate immune responses and communicate with other immunological cells *via* pro-inflammatory or anti-inflammatory cytokines, chemokines, and other bioactive substances. Their functions can be modulatory, protective or deleterious to the surrounding cells, including astrocytes and neurons[12]. Increased microglial activation and enhanced $P2X_7R$ expression are seen in ischemic stroke[1, 4, 9, 49, 53]. Monif *et al.* showed that $P2X_7R$ over-expression is sufficient to drive microglial activation, and ATP stimulation of $P2X_7Rs$ is required for microglial activation[16]. Lu *et al.* recently used a cerebral microemboli model and an *in vitro* culture system to study the roles of microglial activation and $P2X_7$/FasL-Fas signaling following ischemic insult. They proposed an immunoreactive response loop in which microglia-derived FasL exaggerates $P2X_7$-mediated microglial activation and triggers a vicious cycle of neuronal cell death in the ischemic context[47].

However, in early stages of ischemic injury, microglial activation might be protective to the brain *via* $P2X_7Rs$.

During this time, the low concentration of ATP acts as a chemoattractant for microglial recognition and migration[48], directing them to the site of injury[49]. Like macrophages, activated microglia may remove potentially deleterious necrotic cell debris from the ischemic core, promote tissue repair, and thus contribute to neuroprotection[7]. On the other hand, activated microglia release neurotrophic factors *via* activated $P2X_7$Rs and enhance neuronal survival[50]. $P2X_7$R-activated microglia in neuron-microglia co-cultures protect neurons against glutamate toxicity primarily by releasing tumor necrosis factor-α (TNF-α)[50]. Lack of microglia elevates the levels of several brain cytokines and chemokines such as IL-1β, TNF-α, CINC-1 and MCP-1, and increases the severity and volume of injury, indicating that microglia contribute to the endogenous protection against early injury after neonatal stroke[48].

Nevertheless, in the later stages of cerebral ischemia, the high level of ATP stimulates microglial $P2X_7$R over-expression (pore formation)[47] and leads to microglial activation, proliferation[51] and cell death. Over-activated microglia up-regulate the expression of their surface immunomodulatory proteins and release potentially neurotoxic pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α[16, 47]. These pro-inflammatory cytokines boost further microglial activation in an autocrine manner[16, 47] and induce secondary inflammatory injury in the penumbra[22, 50] which is an exaggerated and prolonged inflammatory response that results in continued neuronal cell death (not only damaged cells but also neighboring healthy cells)[10, 16]. Three models were recently proposed to explain the non-classical secretion of mature IL-1β following $P2X_7$R activation[52]. The oldest and simplest model suggests that $P2X_7$R-mediated cytolysis directly triggers rapid caspase-1 activation followed by the release of mature IL-1β[10, 53] depending on the K^+ and Ca^{2+} efflux[54]. The other two models include the involvement of $P2X_7$R-induced microvesicle shedding[24] or lysosome exocytosis from the plasma membrane into the extracellular medium[55].

Activated $P2X_7$ Receptors Modulate Neurotransmitter Release

A series of functional data strongly supports the role of $P2X_7$Rs in information processing in the normal and pathological nervous systems[25]. Several investigations suggest that after cerebral ischemic injury, the extracellularly-accumulated ATP facilitates glutamate and gamma-aminobutyric acid (GABA) release from the terminals of neurons, astrocytes, and cortical microglia upon energy deprivation[3, 56].

The up-regulation and extensive activation of $P2X_7$Rs quickly inhibit glutamate uptake[57], empty the cytoplasmic glutamate store, promote glutamate release and accumulation[42], alter glutamate homeostasis, and trigger cell death[58]. Possibly, the activation of $P2X_7$Rs simultaneously opens both cation channels permeable to large cations and anion channels permeable to organic anions such as *L*-glutamate and *D*-aspartate, mediating rapid non-vesicular glutamate release[26]. In a study of perinatal acute asphyxia, glutamate was verified to be released *via* the $P2X_7$R channel[32].

Although $P2X_7$R channels are not selectively permeable to glutamate, the powerful driving force along the Na^+ and K^+ electrochemical gradient is responsible for significant glutamate efflux. Another underlying mechanism of glutamate release is that the insufficient energy supply and depolarization induced by ischemia cause a direct Na^+ influx, contributing to intracellular Na^+ overload which leads to the opening of voltage-sensitive Na^+ channels[42]. Consequently, endogenous over-activation of $P2X_7$Rs participates in post-ischemic brain injury as an amplifying factor for glutamatergic excitotoxicity[42]. Excessive extracellular glutamate even forms a feedback loop and further down-regulates $P2X_7$Rs[32]. It is noteworthy that this loop may represent a cellular effort to counteract and possibly halt the detrimental outcome of progressive neuronal damage.

GABA efflux is also dependent on the Na^+ and K^+ electrochemical gradients. Cerebral ischemia appears to markedly increase $P2X_7$R-mediated GABA release, which may limit the severity of ischemic brain injury. Augmentation of the excitatory glutamatergic system facilitates the ischemia-induced neuronal damage, whereas augmentation of the inhibitory GABAergic system is neuroprotective. Therefore, the sum of these two opposing mechanisms may determine the infarct size[1].

Therapeutic Potential of $P2X_7$ Receptor Antagonists in Ischemic Brain Injury

Since the up-regulation of the $P2X_7$R system may stimulate

the process of cerebral ischemia, suppressing the deleterious roles of this receptor might be a therapeutic strategy for ameliorating the damage. Increasing numbers of $P2X_7R$ antagonists have been identified and optimized by pharmaceutical companies and academic groups, including KN-62, periodate-oxidized ATP (OxATP), Coomassie brilliant Blue G (BBG), RN-6189, A-740003, and A-438079[59].

Among these, BBG and OxATP are the most widely used in recent studies of $P2X_7Rs$. However, both suffer from relatively poor specificity[51]. OxATP has strong antagonistic effects not only on $P2X_7Rs$, but also on other P2X receptors, and even P2Y receptors. It attenuates pro-inflammatory signaling by mechanisms independent of the expression or activation of P2 receptor subtypes[60]. This occurs in $P2X_7R$-knockout mice, suggesting that one or more other P2 receptors are required[61]. BBG has nanomolar affinity for rat $P2X_7Rs$ and only micromolar affinity for some other P2X receptors.

Of the various $P2X_7R$ antagonists, BBG is the most potent, noncompetitive antagonist. BBG is derived from a blue food-dye, has been shown to be safe in healthy animals, and is approved for foodstuff use. It has low toxicity and can cross the blood-brain barrier[7]. The success of BBG in various animal studies suggests that $P2X_7Rs$ could serve as a therapeutic target for the treatment of neurodegenerative diseases.

Application of BBG reduces the electrical responses, Ca^{2+} influx and neuronal death induced by OGD in neuron cultures and brain slices. Treatment with BBG remarkably reduces the extent of damage, inflammatory responses and learning memory deficits in MCAO and cerebral ischemia/reperfusion injury in the four-vessel occlusion rat model as well[9, 10, 51]. BBG drastically increases neuronal tolerance to mitochondrial dysfunction and glutamate release[10]. It locks the mitochondria and prevents neuronal death by blocking the mitochondrial Ca^{2+} uptake and discharge of the mitochondrial membrane potential.

OxATP has been regarded as a selective $P2X_7R$ antagonist for a considerable time. It is a slowly-equilibrating and irreversible antagonist of $P2X_7Rs$. OxATP has been widely used as a probe to identify $P2X_7R$-mediated actions, especially in the immune system. It markedly attenuates the activation of microglia and interferes with inflammatory signaling processes by decreasing the expression of pro-inflammatory cytokines[3]. Furthermore, OxATP blocks a sustained increase in $[Ca^{2+}]_i$ through the $P2X_7R$ pores and then inhibits the activation of caspase and apoptotic cascades in lipopolysaccharide-induced inflammatory responses[20, 62]. The inhibition of $P2X_7R$-mediated signals by OxATP limits post-ischemic inflammatory responses and confers neuroprotection[62]. OxATP prevents both the up-regulation of $P2X_7Rs$ and neuronal death evoked by their excitotoxic effect[3], but only partially reduces or blocks the cellular damage in organotypic hippocampal cultures[11].

Treatment with OxATP reduces the extent of brain damage in cerebral ischemia/reperfusion injury similar to BBG and A-438079[51]. However, other studies indicated that ischemic injury is exacerbated by OxATP in the rat MCAO model[13]. OxATP administration exacerbates the loss of MAP2-immunoreactivity in neurons in the cerebral cortex and striatum. This discrepancy might be explained by the possibility that $P2X_7Rs$, $P2X_7$-like receptors, and other unknown homologous receptors are expressed in the mouse brain, and ischemic injury may cause compensatory over-expression of $P2X_7$-like receptor mRNAs and induce the opposite effects[13].

KN-62 is another antagonist that may be useful for the pharmacological identification of $P2X_7Rs$[3, 10]. It is also known as a selective CAM-kinase-II inhibitor, a selective antagonist of the human but not the rat $P2X_7R$. KN-62 attenuates the elevation of neuronal nitric oxide synthase serine phosphorylation induced by ischemia in MCAO animals, and plays a neuroprotective role early in ischemia[63].

3-(5-(2,3-dichlorophenyl)-1H-tetrazol-1-yl) methyl pyridine (A-438079) is a newly-discovered, selective antagonist of $P2X_7Rs$ in the brain. A-438079 significantly reduces $P2X_7R$-mediated Ca^{2+} responses and [^{3}H] *D*-aspartate release mediated by BzATP stimulation in HEK293 cells. It also dose-dependently suppresses the production and release of p20-IL-1β, an unconventional 20-kDa form of IL-1β, induced by 4 mmol/L ATP in microglial cell culture[52], indicating the efficacy of this drug in the brain. It is reported that this compound is essentially devoid of activity on other P2 receptors[64].

Treatment with A-438079 correspondingly reduces the extent of brain damage in cerebral ischemia/reperfusion

injury[51]. Rats receiving 3 μg A-438079 have higher neuron survival rates and better motor performance than those receiving 1 μg OxATP. This may be due to the complex action of OxATP on other P2 receptors.

Compared with the other P2X receptor antagonists, PPADS (pyridoxal-phosphate-6- azophenyl-20, 40-disulphonic acid) is a more potent antagonist at P2 receptors. It decreases OGD-evoked glutamate efflux, attenuates glutamatergic excitotoxicity, and thus reduces neuronal death[34, 42, 65]. Therefore, this compound might provide a preferable approach to preventing the long-term ischemic brain injury evoked by glutamate release.

Reactive Blue 2 (RB2) is a sulfonic derivative anthraquinone and a non-selective antagonist of P2 receptors. It does not prevent, but rather promotes $P2X_7R$ expression in microglia and marks their transition from the resting to the reactive state within 24 h after MCAO[4]. This phenomenon occurs after brief treatment with RB2. Long-term RB2 treatment of rats eliminates the effect of $P2X_7R$ blockade in preventing microglial activation and ameliorating the ischemic brain injury in the penumbra. Nevertheless, in remote brain regions, it promotes the expression of $P2X_7Rs$ on reactive microglia, developing defensive and reparative processes[62]. Whether the neuroprotective action of RB2 is solely dependent on the modulation of $P2X_7Rs$ in microglia, or on additional P2 receptors is still unclear.

Conclusion

After cerebral ischemia, the penumbra is damaged but cells within this area can be saved. Consequently, the functional loss associated with stroke can be limited. Strong stimulation of $P2X_7Rs$, which is likely to involve large and nonselective pore formation, leads to cell death of both neurons and certain glial cells, especially microglia. Inhibition of $P2X_7R$ activation to reduce the excitotoxic effect of Ca^{2+} overload, inflammation, and glutamate release would protect against neuronal death and suppress microglial activation.

So far, the physiological functions and the contribution of $P2X_7Rs$ in cerebral ischemic injury remain unclear, even though such information is essential for the evaluation of the $P2X_7R$ as an effective therapeutic target. Whether inhibition of $P2X_7Rs$ has beneficial or harmful effects on brain ischemic injury and whether $P2X_7R$ over-expression drives microglial activation or, conversely, $P2X_7R$ over-expression is a consequence of microglial activation are still not known. The activation of $P2X_7Rs$ to induce neuroinflammation acts as a double-edged sword none the less. Further investigations to discover novel, potent, and more selective $P2X_7R$ antagonists will be critical to evaluate this target's potential therapeutic use, and may provide novel tools both for clinical and research purposes.

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