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REVIEW **Open Access**

Roles of glutamate in brain injuries after subarachnoid hemorrhage

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Summary. Aneurysmal subarachnoid hemorrhage (SAH) is a stroke type with a high rate of mortality and morbidity. Post-SAH brain injury as a determinant of poor outcome is classified into the following two types: early brain injury (EBI) and delayed cerebral ischemia (DCI). EBI consists of various acute brain pathophysiologies that occur within the first 72 hours of SAH in a clinical setting. The underlying mechanisms of DCI are considered to be cerebral vasospasm or microcirculatory disturbance, which develops mostly 4 to 14 days after clinical SAH. Glutamate is the principal neurotransmitter in the central nervous system, but excessive glutamate is known to induce neurotoxicity. Experimental and clinical studies have revealed that excessive glutamates are released after SAH. In addition, many studies have reported the relationships between excessive glutamate release or overactivation of glutamate receptors and excitotoxicity, cortical spreading depolarization, seizure, increased blood-brain barrier permeability, neuroinflammation, microthrombosis formation, microvasospasm, cerebral vasospasm, impairments of brain metabolic supply and demand, impaired neurovascular coupling, and so on, all of which potentially contribute to the development of EBI or DCI. As glutamates always exert their functions through one or more of 4 major receptors of glutamates, it would be valuable to know the mechanisms as to how glutamates cause these pathologies, and the possibility that a glutamate receptor antagonist may block the pathologies. To prevent the mechanistic steps leading to glutamate-mediated neurotoxicity may ameliorate SAH-induced brain injuries and improve the outcomes. This review addresses the current knowledge of glutamate-mediated neurotoxicity, focusing on EBI and DCI after SAH.

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Introduction

In the past several decades, excitotoxicity, a type of neurotoxicity mediated by glutamate, has been at the center of stroke investigation (Suzuki et al., 2022b). Glutamate is the principal neurotransmitter in the adult brain (Suzuki et al., 2022a). Besides the rapid synaptic activation that is critical for neuron-to-neuron communication, glutamate plays crucial roles in neuronal growth, axon guidance, brain development and maturation, and synaptic plasticity in health conditions and diseases (Lai et al., 2014). Ionotropic glutamate receptors such as N-methyl-D-aspartate receptors (NMDARs), α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptors (AMPARs) and kainate receptors, as well as metabotropic glutamate receptors (mGluRs) act as a hub, by detecting extracellular glutamates and processing extracellular glutamate signaling into diverse intracellular outputs in the adult central nervous system (CNS) (Lai et al., 2014). With the emergence of cellular and molecular biology, researchers have elucidated the mechanisms through which the transmission via glutamates and the receptors mediate so many kinds of functional outputs in health conditions and diseases at both levels of microscopic

Abbreviations. AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor; ANXA7, annexin-A7; BBB, blood-brain barrier; CNS, central nervous system; CSD, cortical spreading depolarization; CVS, cerebral vasospasm; DCI, delayed cerebral ischemia; EBI, early brain injury; GABA, γ-aminobutyric acid; GLT-1, glutamate transporter-1; mGluR, metabotropic glutamate receptor; NAM, negative allosteric modulator; NMDAR, N-methyl-D-aspartate receptor; NO, nitric oxide; PI3K, phosphoinositide 3-kinase; PTEN, phosphatase and tensin homolog deleted on chromosome 10; RNF216, ring finger protein 216; SAH, subarachnoid hemorrhage; VASP, vasodilator-stimulated phosphoprotein.

neuron and macroscopic behavior. The mechanisms have important suggestions for research concerning excitotoxicity and its role in neuronal injuries. The identification of distinct intracellular pathways linking glutamate receptor activation to neuronal damage may allow researchers to develop novel therapies targeting a specific harmful signaling pathway without affecting all the signaling pathways downstream of the receptor. The increased specificity would not only reduce the side effects but also expand the therapeutic time window in which a drug can be effectively administered.

Subarachnoid hemorrhage (SAH) by ruptured intracranial aneurysms is a common condition that carries high mortality and morbidity (Feigin et al., 2005). As many as 30,000 of North Americans suffer from SAH annually, accounting for 5 to 10 % of all stroke cases (Boyko et al., 2012). In aneurysmal SAH patients, 10 to 15% of cases are fatal before hospitalization, and about 35% of SAH patients die within the first 30 days of SAH onset (Boyko et al., 2012). The acute pathophysiological events that occur in the brain within the first 72 hours of aneurysmal SAH are collectively called early brain injury (EBI) (Suzuki, 2015). EBI begins to develop within minutes post-SAH: the causes consist of aneurysmal bleeding-induced elevation of the pressure in the cranium and the resultant (transient) global cerebral ischemia, in addition to extravasated blood components, and mechanical brain injuries by concurrent intracerebral hemorrhage and/or acute hydrocephalus (Suzuki, 2015; Suzuki et al., 2020). The pathophysiology of EBI includes the disruption of blood-brain barrier (BBB), microthrombosis formation, early vasoconstriction or microvasospasm, impairments of brain metabolic supply and demand, cortical spreading depolarization (CSD), excitotoxicity, neuroinflammation, free radical reaction, and others, finally leading to neuronal death or apoptosis (Hasegawa et al., 2015; Nishikawa and Suzuki, 2018; Suzuki et al., 2020). More severe EBI is followed by more frequent development of delayed cerebral infarction due to cerebral vasospasm (CVS) or CVS-unrelated delayed cerebral ischemia (DCI) that occurs at days 4 to 14 or later post-SAH (Vergouwen et al., 2011; Suzuki, 2015). Although a great deal of knowledge exists regarding the delayed effects of SAH, the pathophysiology of EBI has not yet been fully understood. Excitotoxicity, the specific type of neurotoxicity induced by glutamates, may be one of missing links between SAH and neuronal death in EBI and DCI, and preventing the mechanistic steps that lead to excitotoxicity might ameliorate SAH-induced brain damage. This review aims to provide a comprehensive summary of the literature on glutamate and excitotoxicity, especially focusing on EBI and DCI after SAH.

Glutamate and excitotoxicity

Brain tissue contains high concentrations of free glutamates intracellularly, serving as a major excitatory amino acid neurotransmitter throughout the CNS, once released into the synaptic cleft (Choi, 2020). Excess glutamates are removed from the extracellular space by astrocytes and endothelial cells with uptake and metabolizing functions, and are exhausted into the circulating blood through diffusion via endothelial cells (Castillo et al., 2016). Glutamates activate NMDARs (GluN1, GluN2A–D, and GluN3A, B), AMPARs (GluA1–4), kainate receptors (GluK1–5), as well as mGluRs (mGluR1–8), and play a pivotal role in synaptic transmission leading to activation of intracellular pathways to regulate cellular functions and to release glutamates in addition to activity-dependent synaptic plasticity (Lai et al., 2014; Serwach and Gruszczynska-Biegala, 2019). NMDARs, AMPARs and kainate receptors are ligand-gated ion channels (Serwach and Gruszczynska-Biegala, 2019).

A number of neurological diseases have been reported to be associated with pathologically elevated levels of extracellular fluid glutamates, including ischemic stroke (Castillo et al., 1996), intracerebral hemorrhage (Castillo et al., 2002), aneurysmal SAH (Helbok et al., 2017), traumatic brain injury (Zauner et al., 1996), glioma (Takano et al., 2001), amyotrophic lateral sclerosis (Andreadou et al., 2008), and many other conditions. This means that CNS insults cause excessive releases of glutamates, leading to overactivation of glutamate receptors (excitotoxicity) (Choi, 2020). Excitotoxicity consists of two components: the first one is an acute, intracellular influx of Na^+ and Cl^-, and subsequently water, causing cell swelling and tissue edema that compress the microvasculature in the surrounding regions resulting in microcirculatory disturbances, and the second one is Ca^{2+}-dependent cell degeneration that occurs somewhat late (Arundine and Tymianski, 2004) (Fig. 1). Post-SAH global cerebral ischemia induces metabolic failure with the disturbance of ionic hemostasis, causing excessive and uncontrolled releases of glutamates within minutes after SAH (Helbok et al., 2017; Suzuki et al., 2021). Cerebral glutamate levels in an acute phase of SAH are already high in patients with neurologically poor status and cerebral edema, and elevated glutamate levels were reported to be an independent predictor of poor outcomes in a clinical setting (Helbok et al., 2017). Aneurysmal SAH causes an increase in proinflammatory cytokines (Okada and Suzuki, 2020), which activate glia to release glutamates, while glutamate reuptake by astrocytes is impaired by proinflammatory cytokines, causing a further increase in extracellular glutamate levels (Zhang et al., 2022). When intracranial cells such as activated astrocytes, microglia and neutrophils synthesize excessive glutamates, the 4 different types of extrasynaptic glutamate receptors, not only on neurons, but also on other cellular components of the neurovascular unit, are overactivated to mediate intracellular Ca^{2+} overload, leading to necrotic, apoptotic and autophagic cell death (Wang and Qin, 2010; Lai et al., 2014; Suzuki et al., 2021). Excessive

intracellular Ca^{2+} entry via the receptors is the primary mediator of excitotoxicity, and intracellular Ca^{2+} overload releases further glutamates, overactivating multiple Ca2+-dependent enzymes (Choi, 2020). That is, there is a positive feedback loop between the stimulation of glutamate receptors and glutamate releases (Suzuki et al., 2021). An alteration in activities of the enzymes impairs mitochondrial energy production and causes increased production of reactive oxygen species to promote lipid peroxidation, leading to cell death (Arundine and Tymianski, 2004; Choi, 2020). Cell damage also causes a release of glutamates (Choi, 2020). In addition, Ca^{2+} influx activates neuronal nitric oxide (NO) and inducible NO synthases in infiltrating neutrophils and endothelial cells, and excessive NO may be involved in free radical-mediated glutamate excitotoxicity, causing caspase-dependent or caspase-independent apoptosis and necrosis (Choi, 2020). Excitotoxicity per se triggers and augments subsequent inflammatory processes: that is, an excitotoxicity-induced release of cytokines and chemokines from the neurovascular unit-constituting cells (neurons, glia, and vascular elements) recruits leukocytes and advances microvascular damage as well as oxidative stresses to continue to destroy brain tissues (Anrather and Iadecola, 2016; Jayaraj et al., 2019). In contrast, neuronal mGluR2 activation may limit a release of neuroprotective neurotransmitter γ-aminobutyric acid (GABA) (Corti et al., 2007), while the expression of both GABA-A and GABA-B receptors decreases after cerebral ischemia, impairing GABA-mediated neurotransmission and exacerbating excitotoxicity (Amantea and Bagetta, 2017). Thus, a key component of post-SAH secondary brain injuries associated with EBI may be glutamate excitotoxicity, which is a pathophysiologic biochemical cascade initiated as a result of the primary brain injury of SAH.

CSD

CSD is slowly self-propagating waves of sustained neuronal and glial depolarization that spread via synapses in all directions from a region of onset (Dreier, 2011). Reportedly, CSD occurs with a relative frequency of approximately 70% of aneurysmal SAH patients (Kramer et al., 2017). CSD may be induced by decreased levels of oxygen and glucose along with increased levels of glutamates in the cerebrospinal and interstitial fluids at global cerebral ischemia by aneurysmal rupture or subsequent post-SAH focal cerebral ischemia (Dreier, 2011). Depolarized neurons or other cells release glutamates, forming a positive feedback loop (Kramer et al., 2017). CSDs usually originate from the boundary hypoperfused zone between injured (presumably already depolarized) and relatively normal brain tissues (von Bornstädt et al., 2015). Once CSD originates, CSDs propagate on the gray matter throughout the surrounding tissues including healthy brain tissues, with elevated extracellular K^+ and glutamates diffusing into the adjacent brain tissues and triggering the same

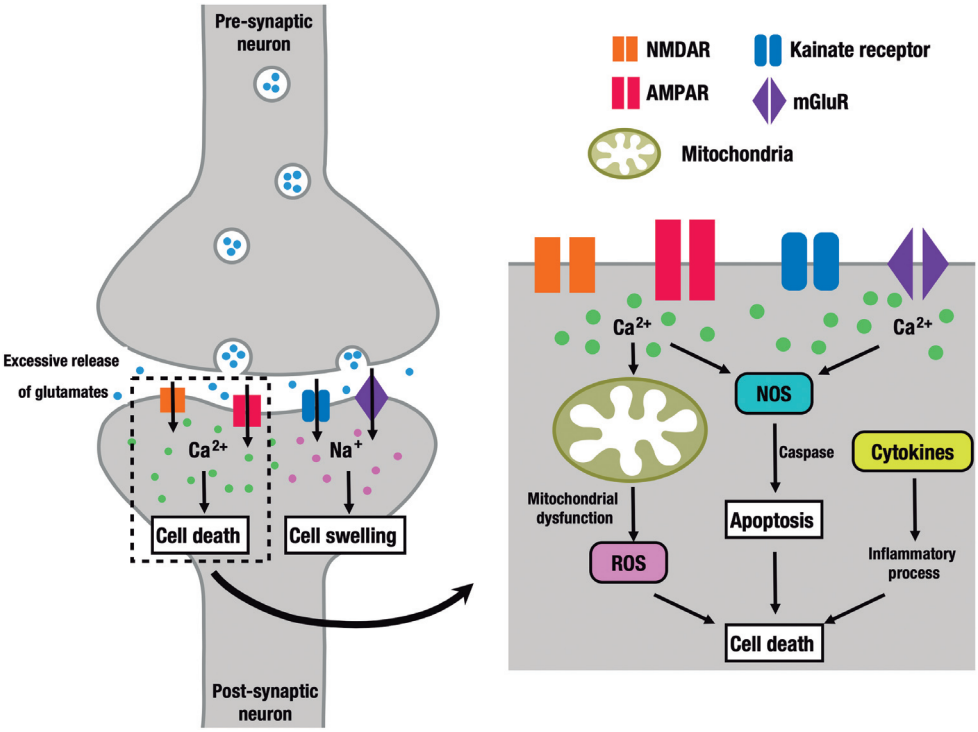

Fig. 1. Excessive release of glutamate after subarachnoid hemorrhage leading to excitotoxity. Overactivated glutamate receptors mediate excessive intracellular influxes of Na^+ and Ca^{2+}, causing cell swelling and cell death. The mechanisms include mitochondrial dysfunction, overproduction of reactive oxygen species (ROS), activation of neuronal nitric oxide synthase (NOS), caspase-dependent or caspase-independent apoptosis, and inflammatory processes. AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; mGluR, metabotropic glutamate receptor; NMDAR, N-methyl-D-aspartate receptor.

depolarization cycles (Chung et al., 2016; Kramer et al., 2017). During CSDs, neuronal membrane potentials approach zero: that is, almost complete depolarization occurs associated with dramatically elevated concentrations of extracellular K^+ and intracellular Na^+ and Ca^{2+}, and therefore precludes the generation of action or postsynaptic potentials, resulting in cortical spreading depression (Chung et al., 2016). If the mechanisms such as Na^+-K^+ pumps do not work to restore a membrane potential, the affected neurons swell from an osmotic shift with an influx of cations (Kramer et al., 2017).

CSD is energetically highly costly and causes heavy metabolic demand, even more than epileptic activities (Chung et al., 2016). After CSDs, repolarization requires intense metabolic activation with increased consumption of O_2 and glucose owing to a compensatory vasodilatory response (normal neurovascular coupling) via a release of NO coupled to CSDs (Sarrafzadeh et al., 2013). However, if the resultant hyperemia and increased oxygen delivery do not meet the demand of energy expenditure, CSD cannot recover (Kramer et al., 2017). In addition, CSDs exert strong arteriolar constriction in ischemic brain tissues by further elevation of extracellular K^+ concentrations that are already high in damaged brain tissues, further worsening the supply–demand mismatch (von Bornstädt et al., 2015; Kramer et al., 2017). Thus, recurrent CSDs in a short time span or CSD clusters emerging spontaneously in metabolically compromised brain tissues after SAH not only impose a tremendous metabolic burden, but also switch the vasodilatory response to an inverse, vasoconstrictive neurovascular coupling, resulting in cortical spreading ischemia, which leads to secondary brain injury or focal cerebral infarction (Bosche et al., 2010) (Fig. 2). The affected ischemic brain tissues are further susceptible to the ongoing spread of CSDs, falling into a vicious cycle (Kramer et al., 2017). CSD clusters also cause excessive releases of glutamates, leading to excitotoxicity (Dreier, 2011; Ashayeri Ahmadabad et al., 2020). In a blood-injection model of SAH in mice, more massive SAH caused more frequent peri-infarct CSDs and vasoconstrictive hypoperfusion responses (Oka et al., 2017). In clinical SAH, frequent occurrence of spontaneous CSDs was associated with the development of delayed cerebral infarction and poor outcomes (Chung et al., 2016; Dreier et al., 2006).

CSD and seizure

Both CSDs (near-complete sustained depolarization of neurons causing spreading depression) and seizures (modest sustained depolarization allowing synchronous, highly frequent neuronal firing) occur after SAH, and can trigger each other (Kramer et al., 2017; Ashayeri Ahmadabad et al., 2020). Glutamates and the receptors may play a central role in the development of both CSD and epileptic activities (Marrannes et al., 1988). In addition, CSDs and epileptic activities have similar toxic effects, such as increased metabolic demand, decreased blood supply due to inverse neurovascular coupling, excitotoxity, activated inflammatory reactions, microthrombosis formation, and BBB disruption with vasogenic brain edema, all of which may aggravate EBI or cause secondary brain injuries (Chung et al., 2016; Suzuki et al., 2022a,b). Toll-like receptor 4 is a common

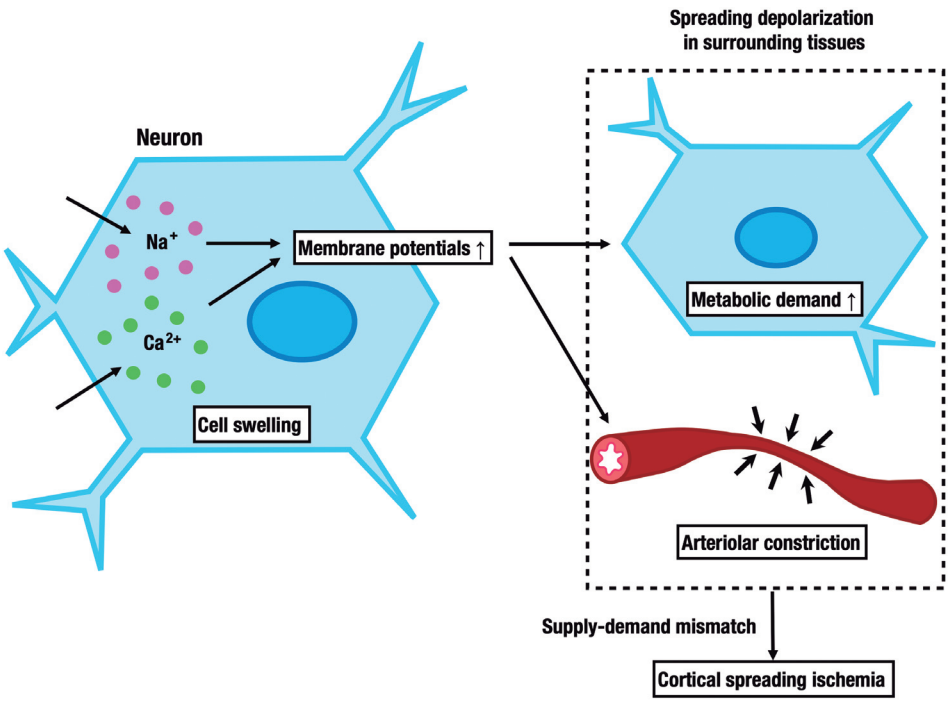

Fig. 2. Cortical spreading depolarization (CSD) and cortical spreading ischemia. CSDs are initiated by glutamate-induced intracellular influxes of Na^+ and Ca^{2+}, propagating throughout the surrounding tissues. In post-subarachnoid hemorrhage brain, CSD clusters cause heavy metabolic demand and strong arteriolar constriction, which worsen the supply-demand mismatch and result in cortical spreading ischemia.

signaling pathway in CSD-induced neuroinflammation in microglia, astrocytes and neurons, leading to neuronal damage (Ashayeri Ahmadabad et al., 2020). Toll-like receptor 4 has also been repeatedly reported to be involved in EBI and DCI after SAH experimentally and clinically (Kawakita et al., 2017; Okada et al., 2019b; Suzuki, 2019; Okada and Suzuki, 2020). Increased permeability of the BBB exposes brain tissues and the extracellular fluid to serum concentrations of ions, leading to more lowered concentrations of extracellular Mg^{2+} and more elevated concentrations of extracellular K^{+}, both of which have been linked to CSD initiation (Kramer et al., 2017). CSD may also contribute to epileptogenesis via BBB disruption after aneurysmal SAH (Dreier et al., 2012).

Glutamate in microthrombosis

Microthrombosis is observed in brain parenchymal microvessels, pial arterioles, large cerebral arteries, as well as cerebral venules and veins, causing brain microcirculatory disturbances and ischemic events after aneurysmal SAH (Fumoto et al., 2019; Khey et al., 2020; Suzuki et al., 2021). A study reported that excessive glutamates caused platelet activation and synthesis of thrombogenic peptides, and that inhibition of mGluR1 or AMPARs attenuated excessive glutamate-mediated microthrombosis formation, possibly both in the subarachnoid space and on the endothelium of brain vasculature after SAH (Gautam et al., 2019; Wang et al., 2020). Meanwhile, platelet-mediated microthrombosis releases glutamates and induces neuronal glutamate receptor dysfunction, mediating excitotoxic brain or neuronal injuries after SAH (Bell et al., 2014). Although glutamates cannot cross the normal BBB, platelet aggregates disrupt the BBB and extravasate to allow neurons to be exposed to excessive glutamates (Bell et al., 2014).

The functional significance of glutamate and microthrombosis in rats at day 6 post-SAH seems to be different from that in EBI at days 1 to 3 post-SAH. At 6 days post-SAH, a rat model of SAH by a prechiasmatic blood injection had platelet-mediated glutamate releases at sites of microthrombosis to cause GluA2 downregulation on neurons, possibly by endocytotic machinery (Hanley and Henley, 2005; Bell et al., 2014). At the same time point in the same animal models, however, a significant decrease of synapses in neurons in the CA1 area was found, not accompanied by ischemia and neuronal death but by GluA1 downregulation and GluA2 upregulation, possibly causing long-term memory impairments (Han et al., 2014).

Glutamate and BBB disruption

BBB disruption is an underlying mechanism of vasogenic brain edema after aneurysmal SAH, and may be developed by multiple mechanisms including endothelial cell apoptosis and disruption of tight junctions (Kanamaru and Suzuki, 2019; Okada et al., 2020). BBB disruption is associated with abluminal and intraparenchymal platelet aggregates, thus releasing glutamates (Bell et al., 2014; Tso and Macdonald, 2014). Glutamates have been demonstrated to increase the permeability of cultured brain endothelial cells via activation of NMDARs, AMPARs, kainate receptors, and mGluRs 1 and 4-8 (Collard et al., 2002; András et al., 2007). Activated mGluRs 1 and 4-8 disturb the BBB by promoting dephosphorylation of vasodilator-stimulated phosphoprotein (VASP) to increase actin filament formation and endothelial cell retraction, resulting in the impairment of cell-cell junctions (Collard et al., 2002; Pula and Krause, 2008).

In animal models, SAH elevated glutamate levels in the cerebrospinal fluid and increased BBB permeability, which was suppressed by inhibiting glutamate releases by the administration of gastrodin (4-hydroxybenzyl alcohol-4-O-β-D-glucopyranoside) (Wang et al., 2019b). An intracerebroventricular injection of glutamate aggravated BBB disruption and cerebral edema, which were suppressed by inhibition of mGluR1: mGluR1 inhibition suppressed glutamate-induced VASP downregulation and inactivation as well as aquaporin-4 upregulation (Zhang et al., 2020). It was also demonstrated that NMDAR activation in brain endothelium led to damage of endothelial cells and that memantine maintained BBB integrity by inactivating NMDAR in experimental SAH (Huang et al., 2015). After experimental SAH, annexin-A7 (ANXA7) protein, which has been shown to regulate glutamate releases, was induced mainly in neurons, and ANXA7 knockdown alleviated BBB disruption and brain edema by reducing glutamate releases (Lin et al., 2019). AMPAR was also shown to play an important role in the development of post-SAH BBB disruption, and an AMPAR antagonist perampanel suppressed post-SAH BBB disruption, brain edema, neurological impairments, as well as epileptic spikes, associated with the suppression of a matricellular protein tenascin-C (Kawakita et al., 2022). As tenascin-C has been involved in the development of various pathologies in EBI and DCI after SAH independently or in cooperation with other matricellular proteins, receptors and molecules, AMPAR may be implicated in other pathologies as well (Kawakita et al., 2019; Shiba and Suzuki, 2019; Nakano et al., 2020; Suzuki et al., 2021). In an endovascular perforation model of SAH in rats, neurological impairments, brain edema, BBB disruption and hippocampal neuronal death were associated with upregulation of phosphatase and tensin homolog deleted on chromosome 10 (PTEN) and alterations of AMPAR subunits at 24 hours post-SAH (Chen et al., 2015). A PTEN inhibitor attenuated BBB disruption and neuronal death, possibly by modulating AMPAR subunits (downregulation of GluA1 and preservation of GluA2 and GluA3) (Chen et al., 2015). AMPARs are not normally Ca^{2+} permeable by virtue of their GluA2 subunits (Doyle et al., 2008), while AMPARs lacking

GluA2 subunits or AMPARs containing an unedited form of GluA2 subunits are rendered permeable to Ca^{2+} (Casillas-Espinosa et al., 2012).

Glutamate and neuronal apoptosis

Cumulative evidence suggests that neuronal apoptosis is one of the major pathological processes involved in EBI after SAH (Cahill et al., 2006; Nakano et al., 2019; Okada et al., 2019a). Under a nonexcitotoxic condition, mGluR1α couples to neuroprotective phosphoinositide 3-kinase (PI3K)-Akt signaling cascades (Chong et al., 2006). Under excitotoxic conditions, however, stimulated NMDARs activate a Ca^{2+}-dependent protease calpain and then induce truncation of mGluR1α, disrupting the link between mGluR1α and PI3K-Akt signaling, while the truncated mGluR1α-mediated intracellular Ca^{2+} increases are maintained to cause Ca^{2+} overloading accompanied by activated NMDAR-mediated Ca^{2+} influxes (Xu et al., 2007). The truncated mGluR1α also induces a release of glutamates and thereby contributes to excitotoxicity, indicating the existence of a positive feedback loop between mGluR1α truncation and excitotoxicity (Xu et al., 2007). GluN2A also may be activated to mediate the pro-survival signaling (Lai et al., 2014), but under tissue acidic conditions such as global cerebral ischemia due to elevated intracranial pressure after aneurysmal rupture, NMDAR activation may be reduced (Giffard et al., 1990).

Under SAH pathology by endovascular perforation in rats, glutamates increased in the cerebrospinal fluid and caused BBB disruption and neuronal apoptosis within 72 hours of SAH (Wang et al., 2019b). Excessive glutamates caused excitotoxicity and subsequently led to a rise in intracellular Ca^{2+}, which plays a crucial role in neuronal death, while intraperitoneal administration of gastrodin inhibited increases in levels of both glutamate and intracellular Ca^{2+}, suppressing neuronal apoptosis at 72 hours post-SAH in rats (Wang et al., 2019b). A calcium-sensitive neuronal NO synthase is physically associated with NMDARs and is activated after SAH-induced glutamate excitotoxicity, but memantine ameliorates neuronal and glial apoptosis through suppression of both activation of NMDARs and upregulation of neuronal NO synthases (Huang et al., 2015). ANXA7 caused neuronal apoptosis by mediating glutamate releases in rats with SAH, and reducing the expression of ANXA7 ameliorated EBI including neuronal apoptosis after SAH (Lin et al., 2019). Neuronal apoptosis induced by excessive glutamate-mediated excitotoxicity, as well as glutamate releases were inhibited by both GluN1/GluN2B negative allosteric modulator (NAM) Ifenprodil and mGluR1 NAM JNJ16259685 through reducing glutamate-induced intracellular Ca^{2+} overload in EBI after experimental SAH (Zhang et al., 2018; Wang et al., 2020). Post-SAH glutamate excitotoxicity was reported to induce activation of calpain and thereby C-terminal truncation of mGluR1α, which suppressed PI3K-Akt signaling and induced caspase-dependent neuronal apoptosis (Wang et al., 2019a). TAT-mGluR1, a fusion peptide consisting of a sequence spanning the calpain cleavage site of mGluR1α and the transactivating regulatory protein transduction domain of human immunodeficiency virus type 1, prevented SAH-induced neuronal apoptosis through inhibiting calpain-mediated mGluR1α truncation (Wang et al., 2019a). At 24 hours post-SAH in a rat model of SAH by a blood injection into the prechiasmatic cistern, knockdown of ring finger protein 216 (RNF216), which regulates a key regulator of neuroinflammation toll-like receptors, prevented post-SAH increases in microglia as well as neuronal apoptosis, and inhibited neurological impairments and brain edema by suppressing post-SAH upregulation of GluA1 and GluA2: the mechanism included an increase in Arc which is a protein coded by the immediate early gene and is closely related to glutamate neurotransmission (Chen et al., 2020). Although the exact roles of AMPARs in neuronal apoptosis have not been fully investigated, the authors demonstrated AMPAR activation in neurons with degeneration after experimental SAH, showing that it is worth further research (Kawakita et al., 2022) (Fig. 3).

In endovascular perforation SAH rats, mGluR5 was expressed in activated microglia (Zhang et al., 2015). An experimental study reported that pharmacological activation of mGluR5 attenuated neurological impairments, brain edema, production of cytokines, microglial activation, and caspase-dependent neuronal apoptosis at 24 hours post-SAH (Zhang et al., 2015). However, it has been reported that activation of mGluR1 and mGluR5 may either aggravate or reduce neuronal damage depending on the context and the nature of toxic insults, although both in vitro and in vivo studies of neuronal death have shown that both mGluR1 and mGluR5 antagonists are consistently protective (Caraci et al., 2012). At 3-5 hours post-SAH in the same model, SAH decreased messenger ribonucleic acid of NMDARs in the hippocampus, possibly providing a neuroprotective mechanism against neuronal death following SAH with moderate hypoperfusion (Bendel et al., 2005).

Microvasospasm and CVS

Angiographic CVS seen on vascular imaging occurs biphasically at ultra-early (within 48 hours of SAH) and delayed phases, and the latter causes DCI (Suzuki et al., 2021). Microvasospasm develops in intraparenchymal perforating arteries, arterioles and capillaries, as well as arterioles on the surface of the brain, playing roles as a cause of EBI and DCI (Suzuki et al., 2021).

Microvasospasm

In normal brain tissues with preserved neurovascular coupling, an increase in neuronal activities induces a

release of a physiological amount of glutamates from neurons, which bind to mGluRs on astrocytes and trigger a mild increase in concentrations of intracellular Ca^{2+} and perivascular K^+, resulting in arteriolar dilatation to increase cerebral blood flow (Tso and Macdonald, 2014). However, post-SAH hemolysis increases basal perivascular K^+ concentrations and decreases NO availability (Suzuki et al., 2022a). In addition, as SAH and associated CSDs release a pathological amount of glutamates, perivascular K^+ concentrations are further and excessively elevated (Suzuki et al., 2022b). Thus, excessive concentrations of perivascular K^+ and impaired availability of NO cause parenchymal arteriolar constriction rather than arteriolar dilatation irrespective of increased metabolism (Suzuki et al., 2022a). This phenomenon is called pathologically inverted neurovascular coupling, which forms the basis of spreading ischemia associated with CSDs after SAH, resulting in mismatch of metabolism and cerebral blood flow, relative cerebral ischemia, and further brain damage that composes one of underlying mechanisms of EBI and DCI (Dreier, 2011; Tso and Macdonald, 2014; Balbi et al., 2017).

Glial and Purkinje cells express mGluR1 and mGluR5, which are activated by glutamates, and then activated mGluR1 and mGluR5 promote the secretion of thromboxane A2, endothelin-1 and 20-hydroxy-eicosatetraenoic acid from these cells, causing microvessel constriction (Mulligan and MacVicar, 2004; Rancillac et al., 2006). Glutamate-mediated activation of mGluR1 and mGluR5 is reported to activate multiple intracellular signaling pathways including phospholipase C, protein kinase C, and mitogen-activated protein kinases, all of which are known to cause vasoconstriction (Niswender and Conn, 2010; Suzuki et al., 2021).

CVS

CVS is one of important causes of DCI or secondary brain injury (Suzuki et al., 2021). CVS is considered to be caused by prolonged arterial smooth muscle cell contraction and impaired vasorelaxation, associated with remodeling of the arterial wall (Suzuki et al., 2021). The underlying mechanisms of CVS consist of inflammatory reactions, modification of extracellular matrix, changes in smooth muscle cell phenotype, injuries or apoptosis of endothelial and smooth muscle cells, and myointimal proliferation (Suzuki et al., 2011). One study suggested that inflammatory cells that infiltrate the perivascular space are capable of secreting multiple humoral factors, many of which have been found to lead to the development of CVS (Garzon-Muvdi et al., 2013). Glutamate is one of the factors, and deactivation of mGluR1 and mGluR5 with S-4-carboxyphenylglycine preserved the phosphorylation status of VASP in endothelial and smooth muscle cells, thereby inhibiting basilar artery CVS in a mouse model of SAH by a blood

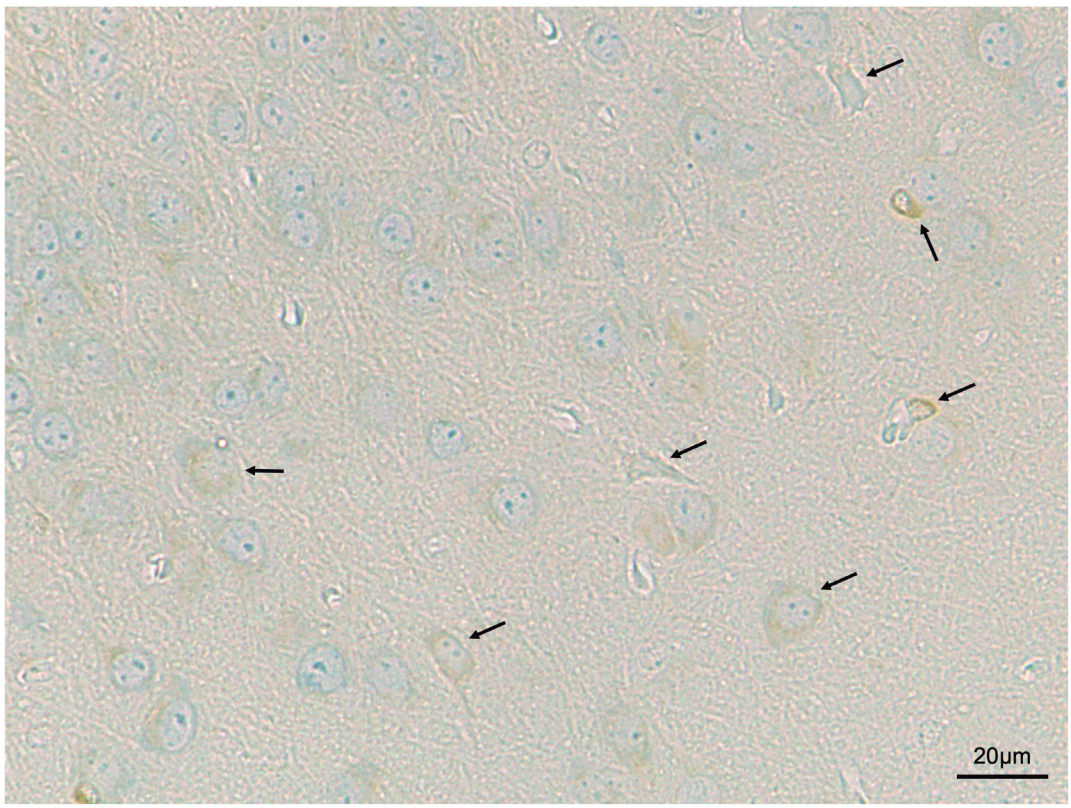

Fig. 3. Representative double immunolabeling of phosphorylated α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (GluA1; brown) and a marker of neuronal nuclei (NeuN; blue) in the left temporal cortex at bregma -1mm at 24 hours after endovascular perforation subarachnoid hemorrhage in mice. Arrow, phosphorylated GluA1- positive degenerated neuron.

injection into the cisterna magna (Garzon-Muvdi et al., 2013). Another study implied that glutamate transporter-1 (GLT-1) played a crucial role in decreasing extracellular glutamate concentrations to inhibit glutamate excitotoxicity, and reported that baicalein treatment prevented CVS, improved neurological function, and reduced mortality through the inhibition of both oxidative stress and glutamate excitotoxicity after experimental SAH (Kuo et al., 2013). The same group also suggested that downregulation of GLT-1 and a significant glutamate surge played a role as a common pathogenic pathway of CVS after experimental SAH, and reported that a peroxisome proliferator-activated receptor agonist rosiglitazone alleviated glutamate elevation, maintained GLT-1 protein expression, and decreased oxidative stress, thus attenuating neurological outcome and mortality after experimental SAH (Lin et al., 2014). Although SAH caused deactivation and downregulation of endothelial NO synthases and VASPs, mGluR1 inhibition by JNJ16259685 prevented SAH-induced basilar artery CVS via facilitating functionalities of endothelial NO synthases and VASPs, associated with the suppression of microthrombosis formation in the cerebral cortex and development of mitochondria-dependent neuronal apoptosis in the CA1 region at day 7 post-SAH (Wang et al., 2020). GYKI-52466, an AMPAR antagonist, was also reported to inhibit vasospasm in a rat femoral artery vasospasm model (Colak et al., 2009).

Memory impairment

Memory impairment was reported to occur in endovascular perforation SAH mice, and was observed at least from 2 to 12 weeks, peaking at 8 weeks post-SAH (Tao et al., 2020). The phenomenon was accompanied by impaired glutamate uptake due to increased expression of histone deacetylase 2 and thereby decreased GLT-1 expression on astrocytes in the hippocampus (Tao et al., 2020). The resultant long-term accumulation of glutamates in the synaptic space results in the dephosphorylation status of GluN2B and GluA1 on the postsynaptic membrane, causing inhibited synaptic excitability and impaired memory function (Tao et al., 2020).

Conclusions

As aforementioned, the function of glutamate is interesting, and the literature suggests that glutamate may be involved in EBI, DCI and other chronic brain injuries after SAH. However, the information is still considerably limited. At present, available data suggest that 1) the onset of SAH and the secondary brain injuries or pathologies lead to an excessive release of glutamate, which overstimulates glutamate receptors and induces excess Ca^{2+} influx through them, followed by Ca^{2+}-dependent activation of death-signaling cascades leading to excitotoxicity; and 2) excitotoxicity, the specific type of neurotoxicity induced by glutamate, may cause EBI and secondary brain injury including DCI after SAH. As a molecular target, glutamates are promising, because they may be involved in multiple underlying mechanisms of EBI and DCI. Considering that glutamates always bind to the receptors to exert their functions, therapeutic strategies targeting glutamate receptors may be a reasonable translational approach for developing new therapies to achieve good functional outcomes after SAH. More experimental and clinical studies are needed to elucidate the relationships between glutamate releases and each pathology of EBI, DCI and other brain injuries, as well as the underlying mechanisms including signaling pathways and interactions with other molecules and enzymes. Further studies may prove that glutamate is a critical regulator of EBI and other brain injuries, and that glutamate is useful as not only a therapeutic target, but also a biomarker to predict, diagnose and monitor progression of EBI, DCI and other brain injuries after SAH and therapeutic effects on them.

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