Thrombin and Brain Recovery After Intracerebral Hemorrhage

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Abstract—Intracerebral hemorrhage (ICH) is a common and often fatal subtype of stroke and produces severe neurological deficits in survivors. At present, there is lack of effective treatments that improve outcome in ICH. A neglected aspect of ICH research is the development of approaches that can be effectively used to improve recovery. Although previous studies have showed that thrombin induces blood–brain barrier leakage, brain edema, and neuronal death after ICH, our recent studies have shown that thrombin may have a role in brain recovery after ICH. An understanding of the mechanisms by which thrombin affects neurogenesis, angiogenesis, and plasticity may facilitate brain recovery after ICH. (Stroke. 2009;40[part 2]:000-000.)

Key Words: angiogenesis ■ cerebral hemorrhage ■ neurogenesis ■ plasticity ■ thrombin

Intracerebral hemorrhage (ICH), from a variety of sources, causes an instantaneous mass effect, disruption of the surrounding brain, and often an early neurological death.1 To date, there are no specific treatments for human ICH. Although thrombin participates in acute brain injury after ICH,1 our recent studies indicate that it also has a role in brain recovery after ICH.2 Evidence suggests that thrombin affects neurogenesis, angiogenesis, and plasticity. This article discusses the pathways activated by thrombin in the brain and their potential role in brain recovery. Clarification of the mechanisms involved in such recovery may be very helpful for developing new therapeutic strategies against ICH-induced brain injury.

Thrombin, Thrombin Receptors, and Signaling Pathways

The essential role of thrombin is to cleave fibrinogen to fibrin. However, other important cellular activities of thrombin, for example, p44/42 mitogen-activated protein kinase (MAPK) activation, appear to be receptor-mediated. Three protease-activated receptors (PARs), PAR-1, PAR-3, and PAR-4, can be activated by thrombin. PARs are 7 transmembrane G protein-coupled receptors that are activated by proteolytic cleavage rather than by ligand binding. PAR-1 expression is found in neurons, astrocytes, oligodendroglial cells, and microglia and there is functional evidence for the presence of PAR-1 on all cell types.

Many intracellular signaling cascades in brain cells can be activated by thrombin.3 Recent studies have demonstrated that thrombin can activate MAPK, phosphoinositol 3-kinase, and p70 S6K.4,5 In rats, p44/42 MAPKs are activated in the brain after intracerebral infusion of thrombin. PD 98059, a specific p44/42 MAPK kinase inhibitor, abolishes thrombin-induced activation of p44/42 MAPKs and also blocks thrombin-induced brain tolerance.4 In addition, thrombin increases brain hypoxia-inducible factor-1α levels through the p44/42 MAPK pathway.6

The phosphoinositol 3-kinase-Akt-mammalian target of rapamycin (mTOR)-p70S6K signaling pathway can be activated by thrombin.5 A phosphoinositol 3-kinase inhibitor, LY-294002, and rapamycin suppressed thrombin-induced DNA synthesis and cell migration.5 As well as evidence that the p44/42 MAPK and phosphoinositol 3-kinase-Akt-mTOR-p70 S6K pathways are activated by thrombin, there is also evidence that these pathways can play a role in neurogenesis. PAR-1 is linked to a wide variety of intracellular signaling cascades.7 Thus, for example, PAR-1 can couple to members of the G12/13, Gq, and Gi families, and, dependent on which G-protein is coupled, it may regulate Rho, inositol 1, 4, 5-trisphosphate, diacylglycerol, adenylate cyclase, and a number of other pathways.3,7

Brain Recovery After Intracerebral Hemorrhage

In earlier studies, we have shown a marked recovery of function over the weeks after ICH in the rat.8 The extent to which this recovery of function after ICH reflects the resumption of normal function by ipsilateral neurons, the assumption of new functions by ipsi- or contralateral neurons or neurogenesis is as yet unknown.

Neurogenesis has been found in animal models after ICH. Recent studies have demonstrated the existence of progenitor cells and their potential for neurogenesis in the subventricular zone, hippocampus dentate gyrus, and cortex of the adult...
mammalian brain. Our recent data showed that neurogenesis occurs after ICH. In that study, cell proliferation marker bromodeoxyuridine and immature neuronal marker doublecortin (DCX) were used. We found that DCX levels in the ipsilateral caudate started to increase as early as 7 days after ICH, peaked at 14 days, and then gradually decreased at 1 month. Immunohistochemistry also demonstrated that DCX immunoreactivity was increased in the ipsilateral subventricular zone and caudate at 2 weeks after ICH. Some DCX-positive cells were bromodeoxyuridine-positive. Temporally, there is some concordance between neurogenesis and improvement in functional outcomes. However, it is still uncertain as to whether ICH-induced neurogenesis contributes to functional recovery.

**Thrombin and Neurogenesis**

The importance of thrombin in modulating brain injury after stroke has become clear. Recent studies have demonstrated a role of thrombin and its receptors in progenitor cells. For example, thrombin stimulates differentiation of bone marrow-derived endothelial progenitor cells. In addition, thrombin enhances the synthesis and secretion of nerve growth factor in glial cells, modulates neurite outgrowth, and stimulates astrocyte proliferation. The effects of thrombin on neurogenesis may, at least in part, be through activation of thrombin receptors. PAR-1 activation stimulates progenitor cell differentiation.

We have also tested the role of thrombin in neurogenesis. One unit of thrombin, which does not cause marked brain injury, was injected into the caudate and it increased DCX levels in the ipsilateral caudate. To examine the effect of thrombin in ICH-induced neurogenesis, a specific thrombin inhibitor, hirudin, was used. Hirudin blocked ICH-induced upregulation of DCX in the ipsilateral caudate.

**Thrombin and Angiogenesis**

Thrombin is a potent promoter of angiogenesis, PAR-1 has an important role in thrombin-induced angiogenesis. Thrombin activates an angiogenic cascade through, at least in part, modulating vascular endothelial growth factor, hypoxia-inducible factor-1, and angiopoietin.

Vascular endothelial growth factor is a specific mitogen of endothelial cells and a strong stimulator of angiogenesis. Thrombin stimulates cells to secrete vascular endothelial growth factor and upregulates vascular endothelial growth factor receptors in endothelial cells. Hypoxia-inducible factor-1, composed of hypoxia-inducible factor-1α and hypoxia-inducible factor-1β subunits, plays an important role in angiogenesis during vascular development. Hypoxia-inducible factor-1α is involved in the regulation of some specific genes, including vascular endothelial growth factor. We have found that intracerebral injection of thrombin causes hypoxia-inducible factor-1α accumulation. In addition, the angiopoietin pathway is modulated by thrombin receptor PAR-1 activation.

**Thrombin and Plasticity**

It is unclear whether thrombin-induced plasticity has a role in brain recovery after ICH. Thrombin is involved in synaptic remodeling and lack of PAR-1 results in learning and memory deficits in mice.

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**Disclosures**

None.

**References**

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