Published in final edited form as:
Stroke. 2009 March ; 40(3 Suppl): S88–S89. doi:10.1161/STROKEAHA.108.533281.

Thrombin and brain recovery after intracerebral hemorrhage

Ya Hua, MD, **Richard F. Keep, PhD**, **Yuxiang Gu, MD, PhD**, and **Guohua Xi, MD**
Department of Neurosurgery, University of Michigan

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 intracerebral hemorrhage (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.

Keywords

angiogenesis; cerebral hemorrhage; plasticity; neurogenesis; thrombin

Introduction

Intracerebral hemorrhage (ICH), from a variety of sources, causes instantaneous mass effect, disruption of surrounding brain, and often an early neurological death [1]. To date there are no specific treatments for human ICH. While thrombin participates in acute brain injury after ICH [1], our recent studies indicate that it also has a role in brain recovery following ICH [2]. Evidence suggests that thrombin affects neurogenesis, angiogenesis and plasticity. This paper 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 kinases (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 seven 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, phosphoinositide 3-kinase (PI3K) 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 MAPKs kinase inhibitor, abolishes

Correspondence and Reprint Requests: Guohua Xi, M.D., R5018 Biomedical Science Research Building, University of Michigan, 109 Zina Pitcher Place, Ann Arbor, Michigan 48109-2200, Telephone: (734) 764-1207, Fax: (734) 763-7322, e-mail: guohuaxi@umich.edu.

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 MAPKs pathway[6].

The PI3K-Akt-mammalian target of rapamycin (mTOR)-p70S6K signaling pathway can be activated by thrombin [5]. A PI3K inhibitor, LY-294002 and rapamycin suppressed thrombin-induced DNA synthesis and cell migration [5]. As well as evidence that the p44/42 MAPK and PI3K-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 $G_{12/13}$, G_q and G_i families, and, dependent on which G-protein is coupled, it may regulate Rho, inositol 1, 4, 5-trisphosphate (IP_3), diacylglycerol, adenylate cyclase and a number of other pathways [3, 7].

Brain recovery following ICH

In earlier studies, we have shown a marked recovery of function over the weeks following 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 adult mammalian brain. Our recent data showed that neurogenesis occurs after ICH [2]. In that study cell proliferation marker bromodeoxyuridine (BrdU) and immature neuronal marker doublecortin (DCX) were used. We found that DCX levels in the ipsilateral caudate started to increase as early as seven days after ICH, peaked at 14 days and then gradually decreased at one month. Immunohistochemistry also demonstrated that DCX immunoreactivity was increased in the ipsilateral subventricular zone and caudate at two weeks after ICH. Some DCX positive cells were BrdU positive. Temporally, there is some concordance between neurogenesis and improvement functional outcomes. However, it is still uncertain as to whether or not ICH-induced neurogenesis contributes to functional recovery.

Thrombin and neurogenesis

The importance of thrombin in modulating brain injury after stroke has become clear [3]. Recent studies have demonstrated a role of thrombin and its receptors in progenitor cells [9]. For example, thrombin stimulates differentiation of bone marrow-derived endothelial progenitor cells [10]. In addition, thrombin enhances the synthesis and secretion of nerve growth factor in glial cells, modulates neurite outgrowth, and stimulates astrocyte proliferation [3]. The effects of thrombin on neurogenesis may, at least in part, be through activation of thrombin receptors. PAR-1 activation stimulates progenitor cell differentiation[10].

We have also tested the role of thrombin in neurogenesis. One unit thrombin, which does not cause marked brain injury, was injected into the caudate and it increased DCX levels in the ipsilateral caudate [2]. 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 [2].

Thrombin and angiogenesis

Thrombin is a potent promoter of angiogenesis. PAR-1 has an important role in thrombin-induced angiogenesis [11]. Thrombin activates an angiogenic cascade through, at least in part, modulating vascular endothelial growth factor (VEGF), hypoxia inducible factor-1 and angiopoietin.

VEGF is a specific mitogen of endothelial cells and a strong stimulator of angiogenesis. Thrombin stimulates cells to secrete VEGF and upregulates VEGF receptors in endothelial cells [11]. Hypoxia inducible factor-1 (HIF-1), composed of HIF-1α and HIF-1β subunits, plays an important role in angiogenesis during vascular development. HIF-1α is involved in the regulation of some specific genes, including VEGF. We have found that intracerebral injection of thrombin causes HIF-1α accumulation [6]. In addition, the angiopoietin pathway is modulated by thrombin receptor PAR-1 activation[12].

Thrombin and plasticity

It is unclear whether or not thrombin-induced plasticity has a role in brain recovery following ICH. Thrombin is involved in synaptic remodeling and lack of PAR-1 results in learning and memory deficits in mice [13, 14].

Acknowledgements

This study was supported by grants NS-017760, NS-039866, and NS-047245 from the National Institutes of Health (NIH), and 0755717Z and 0840016N from American Heart Association (AHA). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH or AHA.

References

1. Xi G, Keep R, Hoff J. Mechanisms of brain injury after intracerebral hemorrhage. Lancet Neurol 2006;5:53–63. [PubMed: 16361023]
2. Yang S, Song S, Hua Y, Nakamura T, Keep RF, Xi G. Effects of thrombin on neurogenesis after intracerebral hemorrhage. Stroke 2008;39:2079–2084. [PubMed: 18436875]
3. Xi G, Reiser G, Keep RF. The role of thrombin and thrombin receptors in ischemic, hemorrhagic and traumatic brain injury: Deleterious or protective? J Neurochem 2003;84:3–9. [PubMed: 12485396]
4. Xi G, Hua Y, Keep RF, Duong HK, Hoff JT. Activation of p44/42 mitogen activated protein kinases in thrombin-induced brain tolerance. Brain Res 2001;895:153–159. [PubMed: 11259772]
5. Cao H, Dronadula N, Rao GN. Thrombin induces expression of FGF-2 via activation of PI3K-Akt-Fra-1 signaling axis leading to DNA synthesis and motility in vascular smooth muscle cells. Am J Physiol Cell Physiol 2006;290:C172–182. [PubMed: 16148030]
6. Hua Y, Keep RF, Hoff JT, Xi G. Thrombin preconditioning attenuates brain edema induced by erythrocytes and iron. J Cereb Blood Flow Metab 2003;23:1448–1454. [PubMed: 14663340]
7. Coughlin SR. Thrombin signalling and protease-activated receptors. Nature 2000;407:258–264. [PubMed: 11001069]
8. Hua Y, Schallert T, Keep RF, Wu J, Hoff JT, Xi G. Behavioral tests after intracerebral hemorrhage in the rat. Stroke 2002;33:2478–2484. [PubMed: 12364741]
9. Smadja DM, Cornet A, Emmerich J, Aiach M, Gaussem P. Endothelial progenitor cells: Characterization, in vitro expansion, and prospects for autologous cell therapy. Cell Biol Toxicol 2007;23:223–239. [PubMed: 17370127]
10. Tarzami ST, Wang G, Li W, Green L, Singh JP. Thrombin and PAR-1 stimulate differentiation of bone marrow-derived endothelial progenitor cells. J Thromb Haemost 2006;4:656–663. [PubMed: 16460448]
11. Tsopanoglou NE, Maragoudakis ME. Inhibition of angiogenesis by small-molecule antagonists of protease-activated receptor-1. Semin Thromb Hemost 2007;33:680–687. [PubMed: 18000795]

12. Smadja DM, Laurendeau I, Avignon C, Vidaud M, Aiach M, Gaussem P. The angiopoietin pathway is modulated by PAR-1 activation on human endothelial progenitor cells. J Thromb Haemost 2006;4:2051–2058. [PubMed: 16803467]

13. Turgeon VL, Houenou LJ. The role of thrombin-like (serine) proteases in the development, plasticity and pathology of the nervous system. Brain Res Rev 1997;25:85–95. [PubMed: 9370052]

14. Almonte AG, Hamill CE, Chhatwal JP, Wingo TS, Barber JA, Lyuboslavsky PN, David Sweatt J, Ressler KJ, White DA, Traynelis SF. Learning and memory deficits in mice lacking protease activated receptor-1. Neurobiol Learn Mem 2007;88:295–304. [PubMed: 17544303]