

## Current perspective on the role of the thrombin receptor in cerebral vasospasm after subarachnoid hemorrhage

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*Current Perspective***Current Perspective on the Role of the Thrombin Receptor in Cerebral Vasospasm After Subarachnoid Hemorrhage**Katsuya Hirano<sup>1,\*</sup> and Mayumi Hirano<sup>1</sup><sup>1</sup>*Division of Molecular Cardiology, Research Institute of Angiocardiology, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan**Received July 28, 2010; Accepted August 23, 2010*

**Abstract.** Cerebral vasospasm is a persistent arterial narrowing typically observed during the 3 – 14 days after subarachnoid hemorrhage (SAH). Vasospasm is frequently associated with ischemic neurological deficits or even death, resulting in a poor prognosis for patients with SAH. However, the mechanism underlying cerebral vasospasm remains elusive, and no effective therapeutic strategies have been established. A large amount of thrombin is produced during SAH. Recent investigations have uncovered a key role of the thrombin receptor in the pathogenesis of cerebral vasospasm. Thrombin has little contractile effect in the normal cerebral artery, but it induces an enhanced and prolonged contraction after SAH, owing to the up-regulation of thrombin receptor PAR<sub>1</sub> (proteinase-activated receptor 1) and the impairment of receptor desensitization in arterial smooth muscle. Thrombin-mediated activation of PAR<sub>1</sub> is an irreversible process, as it is initiated by the proteolytic removal of the N-terminal region. Since the mechanism of receptor desensitization is impaired after SAH, the thrombin-induced contraction irreversibly persists even after terminating thrombin stimulation. Intrathecal administration of a PAR<sub>1</sub> antagonist prevents the PAR<sub>1</sub> up-regulation and the increased reactivity to thrombin. PAR<sub>1</sub> is suggested to play a key role in cerebral vasospasm and may be useful as a therapeutic target for prevention and treatment of cerebral vasospasm.

**Keywords:** desensitization, proteinase-activated receptor, up-regulation, subarachnoid hemorrhage, vasoconstriction

**1. Introduction**

Cerebral vasospasm is associated with both the acute and chronic phases of aneurysmal subarachnoid hemorrhage (SAH) (1). Chronic or delayed cerebral vasospasm typically occurs 3 – 14 days after SAH (1), and it is the most important determinant of morbidity and mortality in the patient with SAH. Vasospasm is diagnosed either angiographically as an arterial narrowing or clinically as delayed ischemic neurological deficits. SAH occurs in about 10 out of 100,000 adults in the general population annually (2). Approximately 70% – 75% of SAH patients develop angiographic cerebral vasospasm, and about

40% of these manifest clinical signs of ischemia (3, 4). Understanding the molecular mechanism(s) underlying cerebral vasospasm is a prerequisite for establishing effective therapeutic strategies for prevention and treatment of the condition, thereby improving the long-term outcome for patients with SAH.

**2. Current understanding of the mechanism of cerebral vasospasm**

The molecular mechanism underlying the development of cerebral vasospasm remains elusive. However, the amount of blood in the subarachnoid space has been shown to correlate with the severity of vasospasm in SAH patients and animal models (5, 6), while the removal of blood clots reverses angiographic vasospasm in animal models (7, 8). As a result, it is conceivable that the presence of blood or the breakdown products of blood are responsible, either directly or indirectly, for the develop-

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ment of cerebral vasospasm (9, 10). These substances, which are undetectable in the normal cerebrospinal fluid but dramatically increased during SAH, and also capable of inducing smooth muscle contraction, are good candidates for possible spasmogens. In line with this, various chemicals and substances derived from blood cells including platelets or the coagulation-fibrinolysis system are suggested to be spasmogens (11). Among them, oxyhemoglobin has been thought to be the primary spasmogen because of its abundance in the cerebrospinal fluid after SAH and its proposed proinflammatory and vasoconstrictive effects (4, 12). However, precisely how oxyhemoglobin causes vasoconstriction and contributes to the development of cerebral vasospasm remains controversial (4). Scavenging nitric oxide or generation of reactive oxygen species appears to contribute to the vasoconstrictive effect of oxyhemoglobin (4). In addition, the metabolites of arachidonic acid, endothelin, inflammation, and disorder of the neuronal regulatory mechanism are suggested to contribute to the pathogenesis of cerebral vasospasm (4). However, their roles still remain to be established. Furthermore, few studies have so far addressed the mechanism responsible for the delayed onset of cerebral vasospasm.

### 3. Current therapeutic strategies for the prevention and treatment of cerebral vasospasm

Because of the lack of knowledge regarding the mechanism of cerebral vasospasm, mechanism-based specific treatments for cerebral vasospasm remain to be established. Currently, hypertensive-hypervolemic-hemodilutional ("triple H") therapy and angioplasty are the only treatments that are partially effective in preventing the ischemic injury caused by vasospasm (12). The aims of these strategies are to maintain the patency of the cerebral artery and to increase blood flow to areas vulnerable to ischemia. With regard to pharmacological treatment, nimodipine (a  $\text{Ca}^{2+}$ -channel blocker), HMG-CoA reductase inhibitors (statins), enoxaparin (a low molecular weight heparin), endothelin-receptor antagonists, and epsilon aminocaproic acid (anti-fibrinolytics) have shown level-1 evidence based on properly designed randomized controlled trials, as defined by the US Preventive Services Task Force ranking system (13), for the treatment of angiographic or clinical vasospasm. In addition, Rho kinase inhibitor, thromboxane  $\text{A}_2$  synthase inhibitor, papaverin, magnesium sulfate, and an adenosine-receptor agonist have also been used for the treatment of vasospasm (11, 13). Among these agents,  $\text{Ca}^{2+}$ -channel blocker, papaverin, magnesium sulfate, and the adenosine receptor agonist are used as vasodilators. Their therapeutic effects are therefore not specific to the spastic arteries.

Statins are known to exert pleiotropic effects in addition to their cholesterol-lowering effect. Among such pleiotropic effects, the up-regulation of endothelial nitric oxide synthase, the inhibition of RhoA activity, and anti-inflammatory effects are all suggested to contribute to the therapeutic effect on cerebral vasospasm (12, 13) because endothelial dysfunction, an increase in the activity of Rho kinase, and inflammation are suggested to be involved in the pathogenesis of cerebral vasospasm (4, 11, 12). Statins may therefore be specific for spastic arteries. Similarly, Rho kinase inhibitors would be expected to dilate spastic vessels, although they could also dilate non-spastic normal arteries (14). Since endothelin-1 and thromboxane  $\text{A}_2$  increase in the cerebrospinal fluid after SAH, an endothelin-receptor antagonist and thromboxane  $\text{A}_2$  synthase inhibitor could be specific for treating vasospasm (4, 11, 12).

### 4. Evidence for involvement of thrombin in the development of cerebral vasospasm

Not only the amount of blood, as mentioned above, but also more specifically the activity of thrombin in the cerebrospinal fluid correlates with the incidence and severity of cerebral vasospasm (15, 16). In contrast, the inhibition of the proteolytic activity of thrombin is associated with the inhibition of cerebral vasospasm (17, 18). Therefore, thrombin is suggested to play an important role in the development of cerebral vasospasm. However, precisely how thrombin contributes to the development of vasospasm remains to be elucidated.

Under physiological conditions, the activity of thrombin is undetectable in the cerebrospinal fluid (19). However, prothrombin, a precursor of thrombin, exists at concentrations of 5–10 nM (19). This amount of prothrombin is capable of generating a level of thrombin high enough to activate thrombin receptors and to induce smooth muscle contraction (20, 21). During SAH, prothrombin derived from blood, in addition to that originally present in the cerebrospinal fluid, could contribute to the generation of thrombin. Therefore, during and after SAH, the total amount of thrombin could reach levels much higher than that required to exert vascular effects (20, 21). As a result, the amount of thrombin dramatically changes in the cerebrospinal fluid during SAH, similar to the changes in oxyhemoglobin. Because of this change in the amount of protein and its potent vasoconstrictive effect, thrombin is relevant as a spasmogen. However, the precise mechanisms by which thrombin causes cerebral vasospasm still remains to be elucidated.

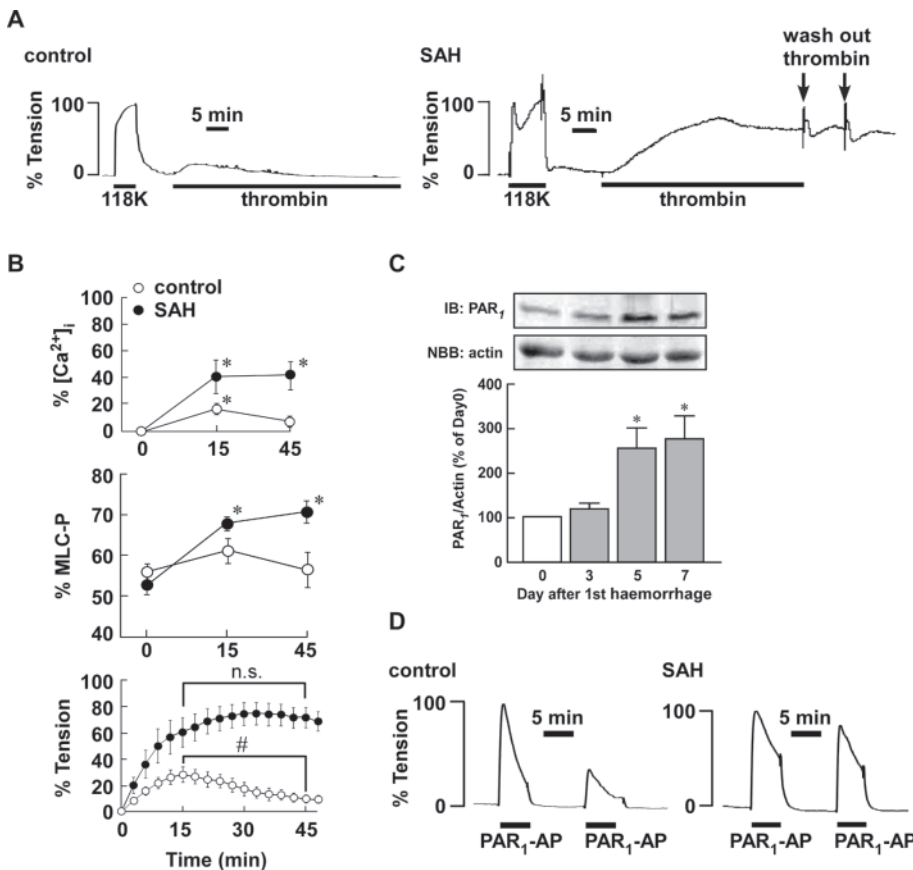
## 5. Up-regulation of the thrombin receptor during subarachnoid hemorrhage

In many normal arteries, including the cerebral artery, thrombin induces little contraction (Fig. 1A) (11, 21), which may undermine the role of thrombin as a spasmogen. However, recent investigations have revealed an increased reactivity to thrombin after SAH, using a rabbit double hemorrhage model (Fig. 1: A and B) (22–24). In the basilar artery isolated from the control animals, thrombin induced only a small contraction even at 10 units/ml (22–24). In contrast, in the basilar artery isolated from the SAH model animals, thrombin induced a significantly enhanced contraction at lower concentrations (22–24). The enhanced reactivity was also observed with a agonist peptide for proteinase-activated receptor (PAR) 1 (PAR<sub>1</sub>-AP), but not for PAR<sub>4</sub> (PAR<sub>4</sub>-AP), thus suggesting that PAR<sub>1</sub> is a major receptor mediating the contractile effect of thrombin after SAH (22, 23). Similar enhancement of the reactivity was also observed with platelet-derived growth factor, an  $\alpha$ -adrenoceptor agonist, and endothelin-1, but not for high K<sup>+</sup>-depolarization or phorbol ester (24, 25). The expression of PAR<sub>1</sub>,  $\alpha$ 1 adrenoceptor, and ET1 receptor

was found to be up-regulated (Fig. 1C) (22–24). Accordingly, the receptor up-regulation is suggested to play an important role in the increased vascular reactivity to agonists. However, the receptor up-regulation could only explain the increased responses to certain spasmogens.

## 6. Impairment of the feedback regulation of the contractile response during subarachnoid hemorrhage

The contractile responses are usually negatively regulated and diminish during the persistent or repeated stimulation with agonist, due to desensitization or tachyphylaxis, respectively. This attenuation of the contractility represents a physiological “feedback” mechanism that protects against both acute and chronic receptor overstimulation, thus helping to prevent the development of cerebral vasospasm. In the basilar artery of the control animals, the contractions induced by thrombin, PAR<sub>1</sub>-AP, endothelin, and phenylephrine gradually decreased after reaching the maximal level of contraction (Fig. 1B) (22, 24). However, after SAH, the contractile response to these agonists was not only enhanced as mentioned above, but also prolonged (Fig. 1B) (22, 24). When the arteries were sequentially stimulated with either PAR<sub>1</sub>-



**Fig. 1.** Enhanced vascular reactivity to thrombin after subarachnoid hemorrhage. A: Representative traces of the contractile response to 118 mM K<sup>+</sup>-depolarization and 1 unit/ml thrombin in the basilar artery isolated from control and animals with subarachnoid hemorrhage (SAH). In SAH, thrombin stimulation was terminated after the contractile response reached the plateau phase (washout). B: Summary of the time course of changes in cytosolic [Ca<sup>2+</sup>]<sub>i</sub>, phosphorylation of myosin light chain (MLC), and tension induced by 1 unit/ml thrombin. C: Immunoblot analysis of the level of PAR<sub>1</sub> expression in the basilar artery on the indicated days after SAH. D: Representative traces of the contractile responses to the sequential stimulation with 100 μM PAR<sub>1</sub>-AP in the basilar artery isolated from control and SAH model animals. The data are the means ± S.E.M. (n = 5–7 in B, n = 3 in C). \**P* < 0.05 vs. time 0 in B and day 0 in C; #*P* < 0.05; n.s., not significantly different. The figures were reproduced and rearranged with permission from the original publications (22, 24).

AP or phenylephrine, the response to the second stimulus was substantially attenuated in the control artery (Fig. 1D) (24). This phenomenon is consistent with tachyphylaxis. After SAH, this tachyphylactic attenuation of the contractile response was compromised, and the reactivity to the second stimulus was maintained (Fig. 1D) (24). These observations thus suggest that the feedback regulation of the contractile response is impaired during SAH, which also underlies the increased vascular reactivity after SAH.

The conversion of the transient response to a prolonged contractile response during SAH is associated with a similar conversion of the  $\text{Ca}^{2+}$ -signal response and the phosphorylation of myosin light chain (Fig. 1B) (22, 24). This observation suggests that the mechanism of feedback regulation is impaired upstream of the  $\text{Ca}^{2+}$  signal, and presumably at the receptor level. In addition, a non-hydrolyzable analogue of GTP,  $\text{GTP}\gamma\text{S}$ , induced a transient contraction in the  $\alpha$ -toxin-permeabilized basilar artery of the control animals, while it induced a sustained response after SAH. This observation suggests that the feedback regulation of the myofilament  $\text{Ca}^{2+}$  sensitivity is also impaired during SAH.

### **7. Impaired receptor inactivation and irreversible contraction induced by thrombin after subarachnoid hemorrhage**

The impairment of the feedback regulation of receptor activity is convincingly suggested at least for  $\text{PAR}_1$ . The activation of  $\text{PAR}_1$  by thrombin is initiated by proteolytic removal of the N-terminal region, which uncovers the region that acts as a tethered ligand and activates the receptor (20, 21). Feedback regulation therefore plays an important role in terminating the activity of the proteolytically activated  $\text{PAR}_1$ . In SAH, thrombin-induced sustained contraction was found to persist even after terminating thrombin stimulation (22, 24). Trypsin is known to remove the ligand region of  $\text{PAR}_1$ , thereby converting the active conformation of  $\text{PAR}_1$  to the inactive conformation (26). The addition of trypsin during the sustained phase of the contraction induced by thrombin decreased the tension to the resting level, while having no effect on the sustained contractions induced by endothelin-1 or  $\text{K}^+$ -depolarization (24). Furthermore, an inhibitor of the GDP-GTP exchange of  $\text{G}_q$  protein also inhibited the thrombin-induced sustained contraction (24). These observations therefore suggest that the persistent contraction is associated with the persistent activation of  $\text{PAR}_1$  and that the feedback inactivation of  $\text{PAR}_1$  is impaired during SAH. The  $\text{G}_q$  inhibitor also inhibited the sustained phase of the contraction induced by endothelin-1 and phenylephrine (24). These observations

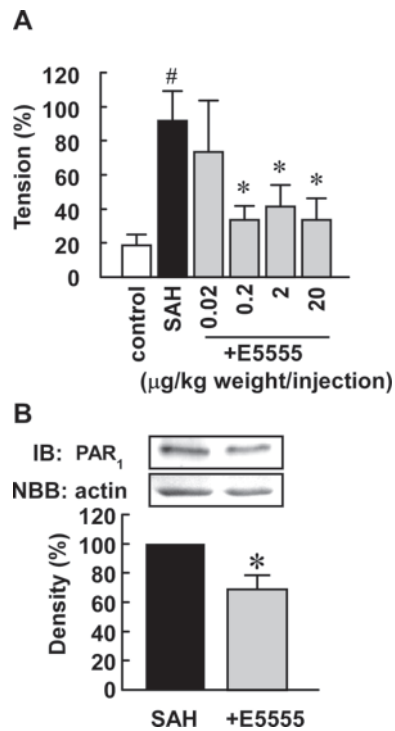
suggest that impairment of the feedback regulation of receptor activity is not limited to  $\text{PAR}_1$  but also extends to other receptors. This general impairment of receptor inactivation may explain the enhanced contractile responses to various spasmogens after SAH. However, this impaired feedback regulation leads to significant effects on the activity of  $\text{PAR}_1$ , thus resulting in irreversible contraction, because this receptor is irreversibly activated in a proteolysis-dependent manner (22, 24).

### **8. Effect of a $\text{PAR}_1$ antagonist on the increased vascular reactivity after subarachnoid hemorrhage**

When autologous blood was heparinized before injection, the enhancement of the contractile response to thrombin was attenuated (22). This suggests that thrombin itself is responsible for enhancing vascular reactivity to thrombin. Several  $\text{PAR}_1$  antagonists with different chemical structures are currently under development, and two of them are under clinical trials for the treatment of ischemic heart diseases as new anti-platelet agents (21, 27).  $\text{PAR}_1$  antagonists do not inhibit the proteolytic activity of thrombin but antagonize the receptor activation by the tethered ligand or by  $\text{PAR}_1$ -AP. As a result,  $\text{PAR}_1$  antagonists inhibit the  $\text{PAR}_1$ -mediated cellular effects of thrombin, without affecting other functions of thrombin such as hemostasis and protein C activation. This mode of action of the  $\text{PAR}_1$  antagonist is therefore beneficial under conditions where a hemorrhage is involved. The intrathecal administration of a  $\text{PAR}_1$  antagonist prevented the up-regulation of  $\text{PAR}_1$  expression and enhancement of the contractile response to thrombin (Fig. 2) (23). This result is consistent with the observations following heparin treatment, suggesting that thrombin-mediated activation of  $\text{PAR}_1$  plays a critical role in upregulating  $\text{PAR}_1$  itself, thereby enhancing the contractile response to thrombin after SAH.  $\text{PAR}_1$  antagonists are thus suggested to provide a mechanism-based new therapeutic strategy for the prevention and treatment of cerebral vasospasm.

### **9. Proposed role of thrombin and $\text{PAR}_1$ in the development of cerebral vasospasm**

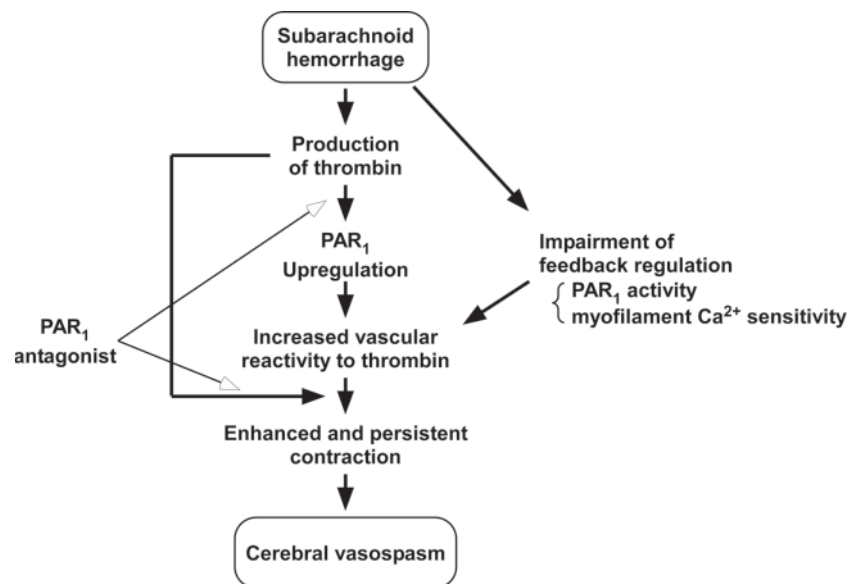
The development of cerebral vasospasm is attributable to either increased production of spasmogens or increased vascular contractility. The above-mentioned observations suggest that both receptor up-regulation and impairment of the feedback regulation of the contractile response contribute to the increased vascular contractility after SAH. The increase in the vascular contractility therefore plays a fundamental role in the induction of cerebral vasospasm by spasmogens and also explains the delayed onset



**Fig. 2.** Preventive effect of a PAR<sub>1</sub> antagonist on the upregulation of PAR<sub>1</sub> and increased reactivity to thrombin after subarachnoid hemorrhage. **A:** Levels of thrombin-induced contraction in the basilar artery isolated from the control and SAH animals with and without intrathecal administration of E5555, a PAR<sub>1</sub> antagonist, at the indicated doses. E5555 was injected twice into the cisterna magna together with autologous blood (23). **B:** The level of PAR<sub>1</sub> protein in the basilar artery of the SAH models with and without 2 µg E5555/kg weight/injection. The data are the means ± S.E.M. (n = 3). \**P* < 0.05 vs. SAH, <sup>#</sup>*P* < 0.05 vs. control. The figures were reproduced and rearranged with permission from the original publication (23).

of cerebral vasospasm. In this context, the recent data suggest a special role of thrombin in the development of cerebral vasospasm, among other substances produced in the cerebrospinal fluid after SAH (22–24). Thrombin functions not only as a spasmogen, but also as a factor that can increase vascular contractility. PAR<sub>1</sub> is therefore a key molecule that mediates both functions of thrombin.

After SAH, thrombin is generated in the subarachnoid space to a level high enough to induce potent vasoconstriction. However, in the normal cerebral artery, thrombin exerts minimal contractile effects. During a SAH, thrombin upregulates the expression of PAR<sub>1</sub> via its activation of PAR<sub>1</sub>, thereby increasing the vascular reactivity to thrombin itself (Fig. 3). Furthermore, the feedback regulation of the contractile response is impaired at the levels of regulation of both the receptor activity and the myofilament Ca<sup>2+</sup> sensitivity (Fig. 3). Under these situations, thrombin can induce an enhanced and prolonged contraction, which irreversibly persists even after terminating the stimulation with thrombin. This persistent contraction appears to mimic the persistent narrowing of the spastic arteries seen in the patients with SAH (Fig. 3). Use of a PAR<sub>1</sub> antagonist not only inhibits the contractile effect of thrombin, but also prevents the upregulation of PAR<sub>1</sub> and the resultant increased reactivity to thrombin (Fig. 3) (23). Targeting PAR<sub>1</sub> can therefore potentially provide an effective therapeutic strategy for both preventing cerebral vasospasm and alleviating the contraction of the spastic artery.



**Fig. 3.** The proposed roles of thrombin and PAR<sub>1</sub> and the sites of action of PAR<sub>1</sub> antagonists during cerebral vasospasm after a subarachnoid hemorrhage.

## 10. Concluding remarks with future perspectives

Recent investigations suggest a critical role for thrombin and PAR<sub>1</sub> in the development of cerebral vasospasm after SAH (Fig. 3). PAR<sub>1</sub> is also suggested to be a useful therapeutic target. PAR<sub>1</sub> antagonists are therefore expected to represent a novel mechanism-based strategy for preventing and treating cerebral vasospasm. However, some questions still remain. First, a PAR<sub>1</sub> antagonist is suggested to prevent PAR<sub>1</sub> upregulation and increased vascular reactivity. However, its effect on the impaired feedback regulation remains to be investigated. The complete restoration of the normal vascular reactivity would require the prevention of both receptor upregulation and impairment of the feedback regulation. The mechanism of the impaired feedback regulation therefore remains to be elucidated. Second, since the thrombin-induced prolonged contraction is maintained by the persistent activity of the proteolytically activated PAR<sub>1</sub> due to the impaired feedback inactivation of PAR<sub>1</sub>, an inverse agonist of PAR<sub>1</sub> may thus be required to inhibit this irreversible contraction. It still remains to be investigated whether any currently available PAR<sub>1</sub> antagonists have an inverse-agonist activity. Finally, it is worth noting that recent clinical trials demonstrated that the endothelin-receptor antagonist clazosentan or the Ca<sup>2+</sup>-channel blocker nicardipine succeeded in preventing arterial narrowing after SAH but failed to improve long-term outcomes (2, 12, 28, 29). This may cause a paradigm shift with respect to the strategy to improve the outcome of SAH patients. Vasospasm may not be the only cause of clinical deterioration. It is suggested that targeting early brain injury and cortical spreading depression is important and beneficial for improving the clinical outcome, rather than merely targeting cerebral vasospasm (2, 28).

It is known that thrombin causes brain damage after cerebral ischemia, hemorrhage, or traumatic injury, especially at high concentrations (30). Therefore, targeting PAR<sub>1</sub> and the use of a PAR<sub>1</sub> antagonist may be a potentially effective strategy for preventing not only the development of cerebral vasospasm, but also ischemic brain damage after SAH. However, such a possibility still remains to be elucidated in future studies.

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