Research Article

Alteration of Basilar Artery Rho-Kinase and Soluble Guanylyl Cyclase Protein Expression in a Rat Model of Cerebral Vasospasm following Subarachnoid Hemorrhage

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Background and Purpose. The vasoconstrictor endothelin-1 (ET-1) has been implicated in the pathogenesis of cerebral vasospasm following subarachnoid hemorrhage (SAH). Previous results showed that CGS 26303, an endothelin converting enzyme (ECE) inhibitor, effectively prevented and reversed arterial narrowing in animal models of SAH. In the present study, we assessed the effect of CGS 26303 on neurological deficits in SAH rats. The involvement of vasoactive pathways downstream of ET-1 signaling in SAH was also investigated. Methods. Sprague-Dawley rats were divided into five groups (n = 6/group): (1) normal control, (2) SAH, (3) SAH+vehicle, (4) SAH+CGS 26303 (prevention), and (5) SAH+CGS 26303 (reversal). SAH was induced by injecting autologous blood into cisterna magna. CGS 26303 (10 mg/kg) was injected intravenously at 1 and 24 hr after the initiation of SAH in the prevention and reversal protocols, respectively. Behavioral changes were assessed at 48 hr after SAH. Protein expression was analyzed by Western blots. Results. Deficits in motor function were obvious in the SAH rats, and CGS 26303 significantly improved the rate of paraplegia. Expressions of rho-kinase-II and membrane-bound protein kinase C-δ and rhoA were significantly increased, while those of soluble guanylyl cyclase α1 and β1 as well as protein kinase G were significantly decreased in the basilar artery of SAH rats. Treatment with CGS 26303 nearly normalized these effects. Conclusions. These results demonstrate that the rhoA/rho-kinase and sGC/cGMP/PKG pathways play pivotal roles in cerebral vasospasm after SAH. It also shows that ECE inhibition is an effective strategy for the treatment of this disease.

1. Introduction

Subarachnoid hemorrhage (SAH) is an important subcategory of stroke due to an unacceptably high mortality rate as well as the severe complications it causes, such as cerebral vasospasm, neurological deficit, and cardiopulmonary abnormality [1]. The potent vasoconstrictor endothelin-1 (ET-1) has been implicated in the pathogenesis of this disease [2]. One strategy to inhibit the biological effect of ET-1 is by means of its receptor antagonists. In fact, various selective and nonselective ET-1 receptor antagonists have been evaluated in animal models of cerebral vasospasm following SAH and in humans with varying degrees of success [3, 4]. An alternative approach to ameliorate the deleterious effects of ET-1 is to suppress the production of this vasoconstrictor by inhibiting endothelin-converting enzyme (ECE), which catalyzes the final step of ET-1 biosynthesis. CGS 26303 is such an inhibitor [5–8], and it has been shown to prevent and reverse cerebral vasospasm in a rabbit model of SAH [9].
The involvement of other vasoactive pathways downstream of ET-1 signaling in SAH is not completely understood. It has been shown that ET-1 potentiates the contraction of cerebrovascular smooth muscles induced by oxyhemoglobin, a blood clot component and major causative factor in cerebral vasospasm, via the protein kinase C (PKC) and rhoA/rho kinase pathways [11]. This is consistent with the finding that the PKC inhibitor staurosporine abolished ET-1-induced contraction in rabbit basilar artery [12]. In addition, lines of evidence accumulated to date have suggested that activation of protein kinase C (PKC) plays a role in the delayed and prolonged contraction of major arteries after activation of proteinkinaseC(PKC)playaroleinthe linesthes evasion accumulating to date has suggested that rhoA/rho kinase pathways [11]. This is consistent with the finding that the PKC inhibitor staurosporine abolished ET-1-induced contraction in rabbit basilar artery [12]. In addition, lines of evidence accumulated to date have suggested that activation of protein kinase C (PKC) plays a role in the delayed and prolonged contraction of major arteries after ET-1 was determined using an ELISA kit according to the manufacturer’s instructions. ET-1 was determined using an ELISA kit according to the manufacturer’s instructions. ET-1 was determined using an ELISA kit according to the manufacturer’s instructions.

2. Materials and Methods

2.1. Materials. Anti-mouse β-actin antibodies and anti-rabbit sGCα1 and sGCβ1 antibodies were obtained from Sigma-Aldrich (St. Louis, MO, USA). Anti-rabbit PKG, anti-mouse PKCδ, anti-rabbit ROCK-III, anti-mouse rhoA, and horseradish peroxidase-labeled goat anti-mouse IgG antibodies were purchased from Abcam (Cambridge, MA, USA), BD Transduction Lab (San Jose, CA, USA), Upstate Biotech (Lake Placid, NY, USA), Santa Cruz Biotech (Santa Cruz, CA, USA), and Chemicon International (Temecula, CA, USA), respectively. CNM protein extraction kits were products of Biochain (Hayward, CA, USA). ET-1 and cGMP ELISA kits were obtained from Assay Designs (Farmingtondale, NY, USA) and Cayman Chemical (Ann Arbor, MI, USA), respectively. CGS 26303 was provided by Dr. Arco Y. Jeng (Novartis Pharmaceuticals, East Hanover, NJ, USA).

2.2. Animal Protocols. All animal procedures were approved by the Kaohsiung Medical University Hospital animal research committee. Thirty male Sprague-Dawley rats (BioLasco, Taiwan) weighing 250–300 g were divided into the following five groups (n = 6/group): Group 1, control animals; Group 2, rats subjected to SAH; Group 3, SAH rats treated with vehicle (0.1 mol/L NaOH/PBS); and Groups 4 and 5, SAH rats treated with CGS 26303 (10 mg/kg, i.v.) at 1 hr (prevention protocol) and 24 hr (reversal protocol) after SAH, respectively. To induce SAH, rats were anesthetized with a mixture of Ketavet (55 mg/kg) and xylazine (9 mg/kg) intraperitoneally (i.p.), and fresh blood (1 mL/kg) was drawn from the central tail artery and injected into the cisterna magna according to a published protocol [8]. The mortality rate after induction of SAH was 15–20%, and it was the same in all of the SAH groups.

2.3. Hemodynamic Measurements. Heart rate and blood pressure were monitored before and after CGS 26303 treatment as well as at 48 hr after the induction of SAH by a tail-cuff method.

2.4. Neurological Assessment. Neurological assessment was performed before and at 48 hr after the induction of SAH. Motor function was quantified by assessment of ambulation and placing and stepping responses using a scoring system published previously and shown in Table 1(a) [26].

2.5. Determination of Plasma ET-1 and Tissue cGMP Levels. Blood was collected in heparin-containing tubes prior to sacrifice. Plasma samples were frozen at −70°C until use. ET-1 was determined using an ELISA kit according to the manufacturer’s instructions. ET-1 was determined using an ELISA kit according to the manufacturer’s instructions. ET-1 was determined using an ELISA kit according to the manufacturer’s instructions.
Table 1: Behavioral changes induced by experimental subarachnoid hemorrhage in the rat.

(a) Scoring system used for motor function assessment

| Motor function          | Behavior                                           | Score |
|-------------------------|----------------------------------------------------|-------|
| Ambulation              |                                                     |       |
| Normal (symmetric and coordinated) |                                                      | 0     |
| Toes flat under the body while walking with ataxia |                                                      | 1     |
| Knuckle-walking         |                                                    | 2     |
| Movement in lower extremities but unable to knuckle walk |                                      | 3     |
| No movement, dragging lower extremities |                               | 4     |
| Placing/stepping reflex |                                                     |       |
| Normal (coordinated lifting and placing response) |                                                      | 0     |
| Weak response           |                                                    | 1     |
| No stepping             |                                                    | 2     |

Ambulation was assessed by walking with lower extremities, while placing/stepping reflex was evaluated by dragging the dorsum of the hind paw over the edge of a surface [10].

(b) Effect of CGS 26303 on behavioral changes induced by experimental subarachnoid hemorrhage in the rat

| Group                 | Ambulation | Placing/stepping reflex | Motor deficit index (MDI) | Paraplegia rate |
|-----------------------|------------|-------------------------|---------------------------|-----------------|
| Normal (no SAH)       | 0.0        | 0.0                     | 0.0                       | 0.0             |
| SAH                   | 1.27 ± 0.18| 1.50 ± 0.13             | 2.36 ± 0.20               | 67              |
| SAH + vehicle         | 1.20 ± 0.13| 1.47 ± 0.13             | 2.27 ± 0.18               | 58              |
| SAH + CGS 26303 (P)   | 0.75 ± 0.13| 0.71 ± 0.17             | 1.33 ± 0.18*              | 30*             |
| SAH + CGS 26303 (R)   | 0.85 ± 0.15| 0.83 ± 0.16             | 1.40 ± 0.15*              | 23*             |

Subarachnoid hemorrhage (SAH) was induced in rats by injecting autologous blood into the cisterna magna. CGS 26303 was administered intravenously at a dose of 10 mg/kg at 1 (prevention protocol, P) or 24 (reversal protocol, R) hr after SAH. The motor function was assessed using the scoring system shown in Table 1(a) and was performed at 48 hr after SAH prior to sacrifice. Six animals were used in each group, and motor function assessment was performed five times for each animal. MDI is the sum of scores from ambulation and placing/stepping reflex. The paraplegia rate is defined as the percentage of rats with MDI ≥ 3 in each group (n = 6). * P < 0.05 versus the SAH group.

3. Results

3.1. General Observations. No statistically significant differences in the body weight, heart rate, or blood pressure were found among the 5 groups at the end of the experiments (results not shown). Visual inspection during the removal of the brain showed that subarachnoid clots had formed and covered the basilar artery in all animals subjected to SAH.

3.2. Neurological Deficit. Using the scoring system shown in Table 1(a), both the ambulation and placing/stepping reflex scores in the SAH and SAH+vehicle groups were significantly higher than in the controls (Table 1(b)). The sum of scores from these two tests is referred to as motor deficit index (MDI). The values of MDI in the SAH and SAH+vehicle groups were 2.36 ± 0.20 and 2.27 ± 0.18, respectively, compared with a score of 0 in the normal control. Treatment with CGS 26303 significantly improved the MDI in the prevention and reversal groups (Table 1(b)). Likewise, paraplegia rate (defined as the percentage of rats with MDI ≥ 3 in each group) was substantially decreased in both the CGS 26303 treatment groups when compared with the SAH animals (Table 1(b)).

3.3. Plasma ET-1 Levels. When compared with controls, plasma ET-1 levels in the SAH group were significantly elevated (Figure 1(a)). Injection with CGS 26303 drastically decreased plasma ET-1 in both the prevention and reversal groups to levels that were not statistically different from the control.

3.4. Tissue Morphometry. The internal elastic lamina in the basilar artery of SAH and SAH+vehicle groups showed substantial corrugation when compared with that obtained from controls (Figure 1(b)). Corrugation was significantly
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Endothelin-1 (pmol/mL)

(a)

Figure 1: Effect of CGS 26303 on plasma ET-1 levels (upper panel) and cross section of basilar artery (lower panel). CGS 26303 was administered at 1 (prevention protocol, P) or 24 (reversal protocol, R) hr after the induction of SAH. Plasma levels of ET-1 were measured by ELISA. Data are mean ± SEM (n = 6/group). ∗, P < 0.05 versus SAH. N: normal control; V: vehicle group. No statistically significant difference was found between the SAH and vehicle groups. The cross sections in the SAH+CGS 26303 group were obtained from animals that underwent the prevention protocol. The arrowheads show the endothelial layer and basal lamina. Scale bars in the first and second rows of the micrograms represent 5 and 0.25 μm, respectively.

less prominent in the SAH+CGS 26303 groups. The cross-sectional areas of basilar artery in the SAH and SAH+vehicle groups were significantly reduced when compared with the control group. Treatment with CGS 26303 significantly attenuated the decrease in both the prevention and reversal groups (results not shown).

3.5. Translocation of PKCδ. It has been shown that PKCδ translocates from the cytosolic compartment to become membrane-bound upon activation. The ratio of PKCδ membrane to cytosolic expression in the basilar artery of the SAH rats was set at 100% as a reference. Only about 60% of PKCδ was membrane-bound in normal animals, treated with CGS 26303 either at 1 or 24 hr after SAH inhibited the translocation of PKCδ to levels similar to that of the normal control (Figure 2).

3.6. RhoA Translocation and ROCK-II Expression in the Basilar Artery. Similar to that seen with PKCδ, rhoA also translocates from the cytosolic compartment to become membrane-bound upon activation. In the basilar artery, membrane-bound rhoA was significantly greater in rats subjected to SAH when compared with the normal control (Figure 3(a)). Treatment with vehicle had no effect, while CGS 26303 significantly reduced membrane-bound rhoA.

The pattern of ROCK-II expression in the basilar artery resembled that observed with levels of membrane-bound PKCδ or rhoA. ROCK-II expression was significantly increased in the SAH and SAH+vehicle groups when compared with controls, and treatment with CGS 26303 normalized the expression of ROCK-II (Figure 3(b)).

3.7. Expression of sGC and Its Downstream cGMP/PKG Pathway in the Basilar Artery. In contrast to an increased expression of ROCK-II in the basilar artery of rats subjected to SAH, the expressions of sGCα1 and sGCβ1 were significantly decreased when compared with the controls (Figure 4). Treatment with CGS 26303 normalized the expressions of
These two enzymes to levels that were not statistically different from the controls (Figure 4).

Consistent with reduced expressions of sGCα1 and sGCβ1 in the basilar artery of SAH rats, the levels of cGMP were also significantly decreased in these animals (Figure 5(a)). Administration of CGS 26303 in both the prevention and reversal protocols significantly attenuated the reduction of cGMP production in the basilar artery of the SAH animals (Figure 5(a)). This increased production of cGMP upon CGS 26303 treatment also resulted in an increased expression of PKG in the basilar artery of the SAH rats which, if untreated, showed a significant reduction in PKG expression when compared with the controls (Figure 5(b)).

3.8. Expressions of ROCK-II, sGCα1, and sGCβ1 in the Brain, Heart, and Lung. It has been shown that ROCK and sGC have a wide distribution in the brain, especially in the cortex, hippocampus, and cerebellum [27, 28]. The expressions of ROCK-II, sGCα1, and sGCβ1 in these regions as well as in the brain stem were investigated. Furthermore, the expressions of these enzymes were examined in the heart and lung, since an increase in the levels of plasma ET-1 was found in the SAH rats and ET-1 was shown to play pathogenic roles in various cardiac and pulmonary diseases.

Unexpectedly, no significant changes in the expressions of ROCK-II, sGCα1, and sGCβ1 were found in these tissues examined in the SAH rats when compared with the control animals, except for an increased expression of ROCK-II in the heart (results not shown). Interestingly, no effects on the expressions of these enzymes were detected upon treatment with CGS 26303 in the SAH animals.

4. Discussion

Cerebral vasospasm following SAH is the leading cause of death and disability after aneurysm rupture. Despite the extensive research and numerous clinical studies conducted, the neurological outcomes in various trials for patients suffering from SAH remain disappointing [29]. Furthermore, assessment of neurological deficits in preclinical studies is scarce. In this regard, the results reported in the present study showing that an ECE inhibitor CGS 26303 significantly improved the motor function index and the rate of paraplegia in the SAH rats are significant findings. In addition, treatment with CGS 26303 decreased the activation of PKCδ and rhoA as well as the expression of rho-kinase, factors thought to contribute to the spastic response, while it concomitantly enhanced components in the sGC/cGMP/PKG pathway. These results suggest that the PKC, rhoA/rho-kinase, and sGC/cGMP/PKG pathways may play important roles in cerebral vasospasm after SAH and that the beneficial effects of CGS 26303 in cerebral vasospasm following SAH might be due to additive influence on all three pathways.

As described herein, the levels of plasma ET-1 were significantly increased with concomitant activation of the PKCδ and rhoA/rho kinase-II pathways in the basilar artery of rats subjected to SAH. Treatment with the ECE inhibitor CGS 26303 normalized plasma ET-1 as well as the expression of the two vasoconstrictive pathways. These results are consistent with the reports showing that oxyhemoglobin is a major causative component of blood clot for cerebral vasospasm following SAH [30, 31] and that ET-1 potentiates the oxyhemoglobin-induced cerebrovascular smooth muscle contraction via the rhoA/rho kinase and PKC pathways [31]. In the present study, the expression of rho kinase-II in the heart, but not in various regions of the brain and the lung, was also activated upon induction of SAH. Interestingly, the expression of ROCK-II in the heart appears to be ET-1-independent as treatment with CGS 26303 had no effect.

In contrast to the activation of the two aforementioned vasoconstrictive pathways, SAH resulted in decreased expression of the vasodilatory pathway sGC/cGMP/PKG (Figures 4 and 5). This pathway has been documented as NO-mediated. However, in a previous study, neither neuronal nor endothelial NO synthase mRNA expression in the brain of the SAH rats was significantly different from that in the brain of the controls and treatment with CGS 26303 had no effect [32]. Nevertheless, it is worth noting that, in addition to its ECE inhibitory activity, CGS 26303 also inhibits the activity of neutral endopeptidase (NEP) [17, 33], which degrades potent vasodilators such as atrial natriuretic peptide (ANP) [34]. In the present study it is possible that an elevated ANP level due to CGS 26303 treatment stimulates the sGC/cGMP/PKG pathway via increased production of NO through the inducible NO synthase as seen in the neonatal rat cardiac myocytes [35].
Figure 3: Inhibitory effect of CGS 26303 on rhoA translocation (a) and ROCK-II expression (b) in the basilar artery. Expression of rhoA in the cytosolic and membrane compartments as well as ROCK-II was determined by Western blot analysis. The ratio of membrane-bound to cytosolic rhoA in the SAH group was set at 100% in (a), whereas the expression of ROCK-II (normalized using β-actin) in the same group was set at 100% in (b). Data are mean ± SEM (n = 6/group). # P < 0.05 versus SAH. No statistically significant difference was found between the SAH and vehicle groups. All groups are identical to those shown in the legend of Figure 1.

Figure 4: Upregulation of sGCα₁ (a) and sGCβ₁ (b) by CGS 26303 in the basilar artery. Expression of sGCα₁ and sGCβ₁ was determined by Western blot analysis and normalized using β-actin. sGCα₁ and sGCβ₁ expressions in the SAH group were set at 100%. Data are mean ± SEM (n = 6/group). * P < 0.05 versus SAH. No statistically significant difference was found between the SAH and vehicle groups. All groups are identical to those shown in the legend of Figure 1.
Figure 5: Increased levels of cGMP (a) and PKG expression (b) by CGS 26303 in the basilar artery. The levels of cGMP were measured by ELISA. Expression of PKG was determined by Western blot analysis. PKG expression in the SAH group was set at 100%. Data are mean ± SEM (n = 6/group). * P < 0.05 versus SAH. No statistically significant difference was found between the SAH and vehicle groups. All groups are identical to those shown in the legend of Figure 1.

5. Conclusion
In summary, this study shows that CGS 26303 reduced the levels of plasma ET-1, improved the motor function index, decreased the activation of the vasoconstrictive PKCδ and rhoA/rho-kinase pathways, and activated the vasodilatory sGC/cGMP/PKG pathway in rats subjected to SAH. It is likely that this compound exerts these beneficial effects via its dual ECE/NEP inhibitory activities. However, confirmation of the utility of CGS 26303 for the treatment of cerebral vasospasm following SAH awaits future clinical studies.

Conflict of Interests
The authors have no conflict of interests or financial disclosures. No part of this paper has been published/presented elsewhere.

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References
[1] V. L. Feigin and M. Findlay, "Advances in subarachnoid hemorrhage," Stroke, vol. 37, no. 2, pp. 305–308, 2006.
[2] C. L. Lin, A. Y. Jeng, S. L. Howng, and A. L. Kwan, "Endothelin and subarachnoid hemorrhage-induced cerebral vasospasm: pathogenesis and treatment," Current Medicinal Chemistry, vol. 11, no. 13, pp. 1779–1791, 2004.
[3] M. Chow, A. S. Dumont, N. F. Kassell et al., "Endothelin receptor antagonists and cerebral vasospasm: an update," Neurosurgery, vol. 51, no. 6, pp. 1333–1342, 2002.
[4] S. G. Keyrouz and M. N. Diringer, "Clinical review: prevention and therapy of vasospasm in subarachnoid hemorrhage," Critical Care, vol. 11, no. 4, p. 220, 2007.
[5] S. De Lombaert, R. D. Ghai, A. Y. Jeng, A. J. Trapani, and R. L. Webb, "Pharmacological profile of a non-peptidic dual inhibitor of neutral endopeptidase 24.11 and endothelin-converting enzyme," Biochemical and Biophysical Research Communications, vol. 204, no. 1, pp. 407–412, 1994.
[6] C. L. Lin, A. S. Dumont, Y. F. Su et al., "Attenuation of experimental subarachnoid hemorrhage by the endothelin-converting enzyme inhibitor CGS 26303," Journal of Neurosurgery, vol. 106, no. 3, pp. 442–448, 2007.
[7] C. L. Lin, A. L. Kwan, A. S. Dumont et al., "Attenuation of experimental subarachnoid hemorrhage-induced increases in circulating intercellular adhesion molecule-1 and cerebral vasospasm by the endothelin-converting enzyme inhibitor CGS 26303," Journal of Neurosurgery, vol. 106, no. 3, pp. 442–448, 2007.
[8] C. L. Lin, H. C. Shih, A. S. Lieu et al., "Attenuation of experimental subarachnoid hemorrhage-induced cerebral vasospasm by the adenosine A2A receptor agonist CGS 21680," Journal of Neurosurgery, vol. 106, no. 3, pp. 436–441, 2007.
[9] A. L. Kwan, C. L. Lin, C. Z. Chang et al., "Continuous intravenous infusion of CGS 26303, an endothelin-converting enzyme inhibitor, prevents and reverses cerebral vasospasm..."
after experimental subarachnoid hemorrhage,” Neurosurgery, vol. 49, no. 2, pp. 422–429, 2001.
[10] H. Shimokawa and M. Rashid, “Development of Rho-kinase inhibitors for cardiovascular medicine,” Trends in Pharmacological Sciences, vol. 28, no. 6, pp. 296–302, 2007.
[11] C. Lan, D. Das, A. Włoskowicz, and B. Vollrath, “Endothelin-1 modulates hemoglobin-mediated signaling in cerebrovascular smooth muscle via RhoA/Rho kinase and protein kinase C,” American Journal of Physiology-Heart and Circulatory Physiology, vol. 286, no. 1, pp. H165–H173, 2004.
[12] A. Y. Zubkova, K. S. Rollins, A. D. Parent, J. Zhang, and R. M. Bryan Jr., “Mechanism of endothelin-1-induced contraction in rabbit basilar artery,” Stroge, vol. 31, no. 2, pp. 526–533, 2000.
[13] C. G. Sobey and F. M. Faraci, “Subarachnoid haemorrhage: what happens to the cerebral arteries?” Clinical and Experimental Pharmacology and Physiology, vol. 25, no. 11, pp. 867–876, 1998.
[14] S. Nishizawa, K. Obara, M. Koide, K. Nakayama, S. Ohta, and T. Yokoyama, “Attenuation of canine cerebral vasospasm after subarachnoid hemorrhage by protein kinase C inhibitors despite augmented phosphorylation of myosin light chain,” Journal of Vascular Research, vol. 40, no. 2, pp. 169–178, 2003.
[15] I. Laher and J. H. Zhang, “Protein kinase C and cerebral vasospasm,” Journal of Cerebral Blood Flow and Metabolism, vol. 21, no. 8, pp. 887–906, 2001.
[16] M. Sugawa, T. Koide, S. Naitoh, M. Takato, T. Matsui, and T. Asano, “Phorbol 12,13-diacetate-induced contraction of the canine basilar artery: role of protein kinase C,” Journal of Cerebral Blood Flow and Metabolism, vol. 11, no. 1, pp. 135–142, 1991.
[17] S. Nishizawa, K. Obara, K. Nakayama et al., “Protein kinase Cα and α are involved in the development of vasospasm after subarachnoid hemorrhage,” European Journal of Pharmacology, vol. 398, no. 1, pp. 113–119, 2000.
[18] S. Nishizawa, K. Obara, M. Koide, K. Nakayama, S. Ohta, and T. Yokoyama, “Attenuation of canine cerebral vasospasm after subarachnoid hemorrhage by protein kinase C inhibitors despite augmented phosphorylation of myosin light chain,” Journal of Vascular Research, vol. 40, no. 2, pp. 169–178, 2003.
[19] J. Hansen-Schwartz, P. Vajkoczy, R. L. Macdonald, R. M. Pluta, and J. H. Zhang, “Cerebral vasospasm: looking beyond vasoconstriction,” Trends in Pharmacological Sciences, vol. 28, no. 6, pp. 252–256, 2007.
[20] H. Shimokawa and A. Takeshita, “Rho-kinase is an important therapeutic target in cardiovascular medicine,” Arteriosclerosis, Thrombosis, and Vascular Biology, vol. 25, no. 9, pp. 1767–1775, 2005.
[21] S. Chrissocholis and C. G. Sobey, “Recent evidence for an involvement of rho-kinase in cerebral vascular disease,” Stroke, vol. 37, no. 8, pp. 2174–2180, 2006.
[22] M. Sato, E. Tani, H. Fujikawa, and K. Kaibuchi, “Involvement of Rho-kinase-mediated phosphorylation of myosin light chain in enhancement of cerebral vasospasm,” Circulation Research, vol. 87, no. 3, pp. 195–200, 2000.
[23] A. Suhardja, “Mechanisms of disease: roles of nitric oxide and endothelin-1 in delayed cerebral vasospasm produced by aneurysmal subarachnoid hemorrhage,” Nature Clinical Practice Cardiovascular Medicine, vol. 1, no. 2, pp. 110–116, 2004.
[24] J. W. Denninger and M. A. Marletta, “Guanylate cyclase and the NO/cGMP signaling pathway,” Biochimica et Biophysica Acta- Bioenergetics, vol. 1411, no. 2-3, pp. 334–350, 1999.
[25] K. A. Lucas, G. M. Pitari, S. Kazeronian et al., “Guanylyl cyclases and signaling by cyclic GMP,” Pharmacological Reviews, vol. 52, no. 3, pp. 375–414, 2000.
[26] M. Kakinohana, S. Nakamura, T. Fuchigami, K. J. Davison, M. Marsala, and K. Sugahara, “Mu and delta, but not kappa, opioid agonists induce spastic paraparesis after a short period of spinal cord ischaemia in rats,” British Journal of Anaesthesia, vol. 96, no. 1, pp. 88–94, 2006.
[27] R. Hashimoto, Y. Nakamura, H. Kosako et al., “Distribution of Rho-kinase in the bovine brain,” Biochemical and Biophysical Research Communications, vol. 263, no. 2, pp. 575–579, 1999.
[28] P. Pifarré, A. García, and G. Mengod, “Species differences in the localization of soluble guanylyl cyclase subunits in monkey and rat brain,” Journal of Comparative Neurology, vol. 500, no. 5, pp. 942–957, 2007.
[29] J. Cahill and J. H. Zhang, “Subarachnoid hemorrhage: is it time for a new direction?” Stroke, vol. 40, supplement 3, pp. S86–S87, 2009.
[30] R. L. Macdonald and B. K. A. Weir, “A review of hemoglobin and the pathogenesis of cerebral vasospasm,” Stroke, vol. 22, no. 8, pp. 971–982, 1991.
[31] G. Wickman, C. Lan, and B. Vollrath, “Functional roles of the Rho/Rho kinase pathway and protein kinase C in the regulation of cerebrovascular constriction mediated by hemoglobin: relevance to subarachnoid hemorrhage and vasospasm,” Circulation Research, vol. 92, no. 7, pp. 809–816, 2003.
[32] C. P. Yen, S. C. Chen, T. K. Lin et al., “CGS 26303 upregulates mRNA expression of heme oxygenase-1 in brain tissue of rats subjected to experimental subarachnoid hemorrhage,” Journal of Cardiovascular Pharmacology, vol. 44, supplement 1, pp. S474–S478, 2004.
[33] I. Tikkanen, T. Tikkanen, Z. Cao et al., “Combined inhibition of neutral endopeptidase with angiotensin converting enzyme or endothelin converting enzyme in experimental diabetes,” Journal of Hypertension, vol. 20, no. 4, pp. 707–714, 2002.
[34] J. L. Sonnenberg, Y. Sakane, A. Y. Jeng et al., “Identification of protease 3.4.24.11 as the major atrial natriuretic factor degrading enzyme in the rat kidney,” Peptides, vol. 9, no. 1, pp. 173–180, 1988.
[35] K. Yamamoto, U. Ikeda, and K. Shimada, “Natriuretic peptides modulate nitric oxide synthesis in cytokine-stimulated cardiac myocytes,” Journal of Molecular and Cellular Cardiology, vol. 29, no. 9, pp. 2375–2382, 1997.