

## Open Access

# Recent Insights Into the Mechanisms of Vasospastic Angina

Sang-Yong Yoo, MD<sup>1</sup> and Jang-Young Kim, MD<sup>2</sup>

<sup>1</sup>Division of Cardiology, Department of Internal Medicine, University of Ulsan College of Medicine, Gangneung Asan Hospital, Gangneung,

<sup>2</sup>Division of Cardiology, Department of Internal Medicine, Wonju College of Medicine, Yonsei University, Wonju, Korea

## ABSTRACT

Coronary artery spasm plays an important role in the pathogenesis of many types of ischemic heart disease, not only in vasospastic angina but also in myocardial infarction and sudden death, particularly in the Asian population. Patients with vasospastic angina are known to have defective endothelial function due to reduced nitric oxide bioavailability. Moreover, markers of oxidative stress and plasma levels of C-reactive protein are elevated. Smoking, polymorphisms of endothelial nitric oxide synthetase (eNOS), and low-grade inflammation have been regarded as the most important risk factors for vasospastic angina. The recent body of evidence indicates that RhoA and its downstream effector, ROCK/Rho-kinase, are associated with hypercontraction of vascular smooth muscle of the coronary artery and regulation of eNOS activity. Thus, endothelial dysfunction through abnormalities of eNOS and enhanced contractility of vascular smooth muscle in coronary artery segments are considered major mechanisms in vasospastic angina. However, the precise mechanisms for coronary vasospasm are not well understood. This article will review current understanding of the mechanism of coronary artery spasm. (**Korean Circ J 2009;39: 505-511**)

**KEY WORDS:** Coronary vasospasm; Vascular smooth muscle; Vascular endothelium.

## Introduction

Although prevalence of vasospastic angina appears to be on the decline throughout the world, probably due to widespread use of calcium antagonists,<sup>1)</sup> resting chest pain in a relatively young patient during the early morning hours should raise the suspicion of vasospastic angina, particularly when occurring during sobriety. Although the role of coronary vasoconstriction was originally discovered in “variant angina”, there is convincing evidence that various types of coronary constriction play a role in major ischemic heart disease; moreover, despite treatment with calcium channel blockers and nitrate, recurrent episodes of angina attack in patients with vasospastic angina are frequently observed, whereas sudden cardiac

death or non-fatal myocardial infarction is rare.<sup>2)</sup> Therefore, clarification of the exact mechanisms of coronary artery spasm for aid in development of novel and fundamental therapeutic targets is very important.

Myocardial ischemia caused by coronary artery spasm can develop in both large epicardial and small distal coronary arteries. Reduction in epicardial coronary artery diameter in response to ergonovine is usually diffuse, less than 30%, in patients who do not have vasospastic angina.<sup>3)</sup> This constrictor response is considered to be within “physiological” range. Although constrictor stimuli capable of inducing myocardial ischemia do exist in a normal coronary artery, they appear to predominantly exert their action on small distal vessels and secondary and tertiary branches, rather than on proximal epicardial segments, where spasm occurs more frequently.<sup>4)</sup>

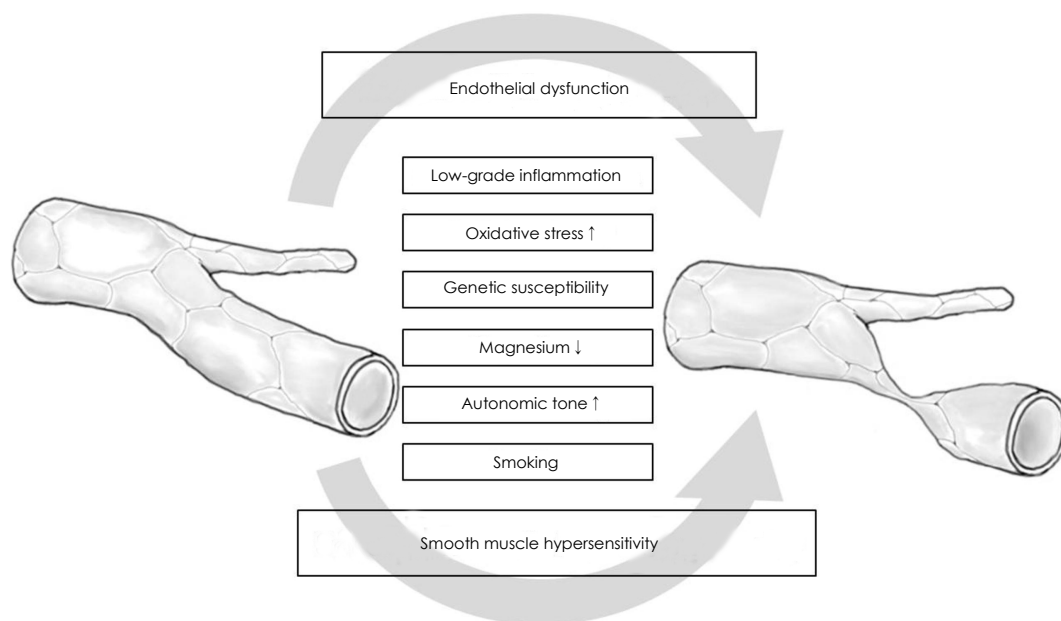
Recent studies have identified a number of mechanisms and precipitating factors that may play a role in pathogenesis of vasospastic angina (Fig. 1). These include endothelial dysfunction, smooth muscle hypersensitivity, increased autonomic tone, increased oxidative stress, decreased magnesium, low-grade inflammation, and genetic susceptibility. Pathologic mechanisms of coronary vasospasm causing myocardial ischemia may be multiple, or may have interactions with each other. For the com-

**Correspondence:** Sang-Yong Yoo, MD, Division of Cardiology, Department of Internal Medicine, University of Ulsan College of Medicine, Gangneung Asan Hospital, 415 Bangdong-ri, Sacheon-myeon, Gangneung 210-711, Korea

Tel: 82-33-610-3130, Fax: 82-33-641-8130

E-mail: bovio@naver.com

© This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.



**Fig. 1.** Mechanisms and precipitating factors of vasospastic angina. A number of mechanisms and precipitating factors may play a role in pathogenesis of vasospastic angina. Endothelial nitric oxide activity is reduced and markers of low-grade inflammation and oxidative stress are elevated in patients with vasospastic angina. Magnesium deficiency may be related to coronary vasospasm in some patients, and smoking is regarded as an independent risk factor for development of vasospastic angina. Recently, hyper-contraction of smooth muscle of the coronary artery in the presence of increased  $\text{Ca}^{2+}$  sensitivity has been considered an important molecular mechanism of coronary vasospasm. Pathologic mechanisms of coronary artery vasospasm causing myocardial ischemia might be multiple, or might interact with each other.

prehensive explanation, the authors divided mechanisms into two parts; the first is dysfunctional vascular endothelium, and the other is enhanced vascular tone of local smooth muscle. In the present review, we will discuss our point of view, focusing on these two scenarios and recent insights into the pathophysiology of vasospastic angina.

## Dysfunctional Vascular Endothelium

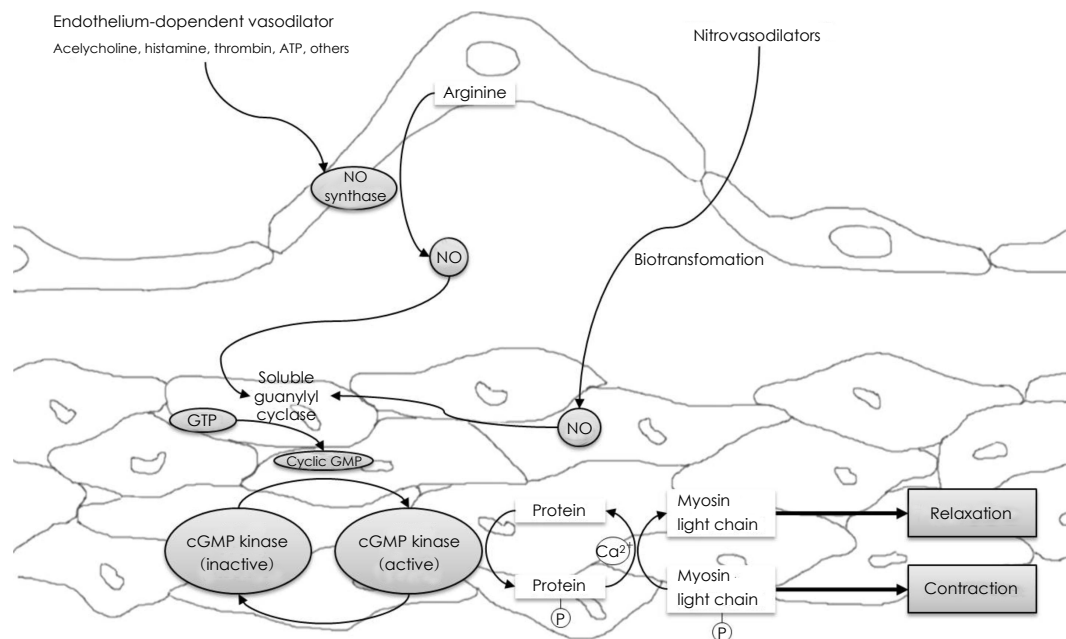
### Vascular endothelium and nitric oxide

The endothelium is a single-cell lining covering the internal surface of blood vessels, cardiac valves, and numerous body cavities; it is composed of approximately  $1$  to  $6 \times 10^{13}$  cells, and covers a surface area equivalent to about six tennis courts.<sup>5)</sup> Yet, as recently as the first half of the 20th century, endothelial cells were simply regarded as barriers to blood flow. However, it was recently recognized that the vascular endothelium, which is strategically located to “sense” changes in hemodynamic forces and blood-borne signals and to “respond” by releasing a number of vasoactive substances, is a multifunctional organ with complex metabolic capabilities, including regulation of vascular tone, cellular adhesion, thromboresistance, smooth muscle cell proliferation, and vessel wall inflammation.<sup>5,6)</sup> Therefore, the integrity of the vascular endothelium and a well-balanced release of numerous vasoactive substances are considered essential to normal vascular physiology, and its dysfunction can be critical to pathogenesis of vascular disease.<sup>7)</sup>

Vascular endothelium undergoes a constant process of injury and repair in response to mechanical and chemical injury. At the site of tissue vascularization, endothelial cells can originate either from adjacent preexisting blood vessels or from recruitment of bone marrow-derived circulating endothelial progenitor cells (EPCs). Repair with EPCs is associated with normalization of endothelial function at the site of injury,<sup>8,9)</sup> and impairment of the ability to participate in vascular repair has been demonstrated in an animal model of decreased nitric oxide (NO) activity.<sup>10)</sup> NO, synthesized from L-arginine by an endothelial isoform of NO synthase (eNOS), is a key endothelium-derived relaxing factor (EDRF), and plays a pivotal role in maintenance of vascular homeostasis (Fig. 2).<sup>11)</sup> NO synthesis is specifically blocked by L-monomethyl-arginine (L-NMMA).<sup>12)</sup> Although NO was discovered as a vasodilator, NO has multiple antiatherogenic roles, including an anti-inflammatory, antithrombotic, antiproliferative, and antioxidant effect.<sup>13)</sup> Thus, vascular endothelial dysfunction may be associated with a relative deficiency of EPCs and/or decreased NO activity in vascular repair.<sup>14,15)</sup>

### Atherosclerosis and vasospastic angina

Endothelial dysfunction, as characterized by impairment of endothelium-dependent relaxation, and thereby reduced eNOS-derived NO bioactivity, is a critical step in development of atherosclerosis.<sup>16,17)</sup> It is well known that atherosclerotic disease affecting large coronary ar-



**Fig. 2.** Endothelium-dependent relaxation of smooth muscle. Nitric oxide (NO) is synthesized in endothelial cells from L-arginine by endothelial NO synthase. Following uptake into vascular smooth muscle cells, NO stimulates soluble guanylyl cyclase to form cyclic guanosine monophosphate (cGMP). cGMP activates cGMP-dependent protein kinase, resulting in protein phosphorylation, myosin light chain dephosphorylation, and relaxation (Adapted and modified from reference 67). GTP: guanosine triphosphate.

teries can alter their vasomotor tone and reactivity.<sup>18-20</sup> MacAlpin<sup>21</sup> reported 88% localization of coronary artery spasm at the site of an atherosclerotic lesion, and some studies using intravascular ultrasound have revealed atherosclerotic plaques in just about any spastic segment.<sup>22</sup> A report using histological evaluation of coronary plaques in patients with vasospastic angina also revealed evidence of inflammatory cell infiltration.<sup>23</sup> Using a porcine model of atherosclerosis with endothelium removal and high-cholesterol feeding, intracoronary histamine or serotonin induced coronary artery spasm at the site of the atherosclerotic lesion, revealing a close topological correlation between the spastic site and the atherosclerotic lesion.<sup>24,25</sup> These results provided evidence of a close relationship between coronary artery spasm and coronary atherosclerosis.

Although presence of atherosclerosis may impair endothelium-dependent dilation to acetylcholine (ACh), ACh has a major direct constrictor action on smooth muscle at high doses in subjects with angiographically normal arteries.<sup>26</sup> In addition, a discrepancy exists between ACh-induced coronary spasm and spontaneous spasm or that induced by ergonovine or serotonin.<sup>27,28</sup> ACh-induced coronary spasm is relatively diffuse, involving all coronary segments, whereas spontaneous spasm or that induced by ergonovine or serotonin occurs at a given coronary segment. In addition, observation of responses to substance P, a pure endothelium-dependent vasodilator, has led some investigators to the conclusion that endothelial dysfunction does not occur in patients with vasospastic angina.<sup>29,30</sup> Therefore, because it lacks the ability to dis-

criminate the role of endothelial dysfunction from that of vascular smooth muscle hypersensitivity, ACh may not be a suitable agent for evaluation of endothelial dysfunction of coronary arteries.<sup>26</sup>

### Decreased nitric oxide activity and vasospastic angina

ACh, serotonin, or histamine, all endothelium-dependent vasodilators by release of NO, induce coronary dilation in young healthy subjects, but cause paradoxical vasoconstriction in patients with coronary atherosclerosis.<sup>7,12,31</sup> Therefore, endothelial function of the coronary arteries has been evaluated using intracoronary or intravenous injection of endothelium-dependent vasodilators, such as ACh. Coronary arteries of patients with vasospastic angina are highly sensitive to the vasoconstrictor effect of intracoronary ACh injection.<sup>32</sup>

In addition, nitrates, including nitroglycerin, are endothelium-independent vasodilators that cause vasodilation by way of their conversion to NO; the response of nitroglycerin is markedly increased in spasm arteries compared to control arteries,<sup>33</sup> an indication that removal of basal NO-mediated vasodilatation leads to supersensitivity to an exogenous nitrovasodilator. Thus, deficiency of both basal and ACh-induced NO activity in spasm arteries in patients with vasospastic angina is quite possible.<sup>33-35</sup>

Deficiency of NO activity may be due either to a decrease in production or an increase in degradation of NO. Studies using a specific NO synthase inhibitor, L-NMMA, have already reported endothelial dysfunction

with decreased endothelial NO production in patients with vasospastic angina.<sup>33)</sup> A strong association of polymorphisms of T-786C in the 5'-flanking region and Glu-298Asp in exon7 of the eNOS gene with vasospastic angina and compromised endothelial NO production has recently been demonstrated.<sup>36)37)</sup>

#### Increased oxidative stress and vasospastic angina

Oxygen free radicals can directly damage endothelial cells and degrade NO, leading to vasoconstriction.<sup>12)</sup> However, direct evidence of the role of oxidative stress in patients with vasospastic angina is lacking. Epidemiological studies have shown that cigarette smoking is a major risk factor for vasospastic angina.<sup>38)</sup> In animal models, cigarette smoke, which contains large amounts of oxygen free radicals, suppressed ACh-induced endothelium-dependent vasodilatation, and this suppression was prevented by antioxidants or superoxide dismutase.<sup>39-41)</sup> Patients with vasospastic angina have low antioxidant concentrations, and administration of antioxidants (such as vitamin C and E) has been shown to improve endothelium-dependent vasodilatation.<sup>42)43)</sup> Therefore, antioxidant therapy, in addition to conventional treatment, will be beneficial in treatment of vasospastic angina. The antioxidant activity of high-density lipoprotein is known to protect low density lipoprotein-cholesterol (LDL) against oxidative modification via paraoxonase by preventing accumulation of lipid peroxides.<sup>44)</sup> A significant association between vasospastic angina and the paraoxonase gene Glu192Arg (Q182R) polymorphism was recently reported.<sup>45)</sup>

#### Imbalance between vasoconstrictor and vasodilator

In addition to a decrease in production or an increase in degradation of NO, resulting in deficiency of NO activity, there can be an imbalance between endothelium-produced vasodilator factors (i.e., prostacyclin, NO) and vasoconstrictor factors (i.e., endothelin, angiotensin II) that favors the latter.<sup>46)</sup> NO is known to suppress production of endothelin-1 and angiotensin II, which are potent vasoconstrictors and proliferators of vascular smooth muscle, and deficiency of NO may enhance synthesis of these vasoconstrictors.<sup>47)48)</sup> Endothelium also produces vasodilators, such as prostacyclin<sup>49)</sup> or hyperpolarizing factor,<sup>50)</sup> and vasoconstrictors, such as endothelins<sup>51)</sup> or endothelium-dependent constricting factors.<sup>52)</sup> Therefore, it is possible that decrease of vasodilators or increase of vasoconstrictors may also be involved in pathogenesis of vasospastic angina.

#### Chronic low-grade inflammation and vasospastic angina

Since 1978, when Lewis and co-workers first reported on a case connecting inflammation and vasospastic angina,<sup>53)</sup>

the body of evidence suggesting a relationship between inflammation and coronary vasospasm has grown.<sup>54)</sup>

Circulating plasma levels of P-selectin, E-selectin, and intercellular cell adhesion molecule-1 were elevated in patients with vasospastic angina, indicating an association of endothelial damage and inflammatory reaction with coronary vasospasm.<sup>55)56)</sup> Hung et al.<sup>57)</sup> prospectively investigated the association of high-sensitivity C-reactive protein (CRP), a sensitive marker of inflammation, with coronary vasospasm and no hemodynamically significant coronary artery disease in a sample of 428 patients who underwent coronary angiography. Results showed that high-sensitivity CRP level was independently associated with a diagnosis of coronary vasospastic angina. These observations indicate that chronic low-grade inflammation plays an important role in pathogenesis of coronary vasospasm. However, much more research regarding the direct association between inflammation and vasospastic angina is obviously needed.

### Controversies Regarding Dysfunctional Endothelium Versus Enhanced Smooth Muscle Contractility

Shimokawa<sup>58)</sup> reasoned that coronary artery spasm is caused primarily by hypercontraction of coronary smooth muscle cells, although endothelial dysfunction may be important in induction of early atherosclerotic changes of the coronary artery, favoring occurrence of a spasm. Reasons for this notion are as follows: First, coronary artery spasm occurs at a given site within the atherosclerotic coronary artery, whereas endothelial dysfunction appears to be more generalized throughout the epicardial coronary arteries or in blood vessels throughout the body. Second, vasodilating responses to bradykinin or substance P, both of which are endothelium-dependent vasodilators, are fairly well preserved at the spastic coronary segment in patients with vasospastic angina. Third, the coronary atherosclerotic lesion remains at the spastic site, even after spontaneous remission of the spastic activity of the coronary artery in patients with vasospastic angina. Fourth, although long-term treatment with fish oils is known to improve endothelial vasodilator function, vasospastic activity in patients with vasospastic angina can last even longer after long-term treatment with eicosapentaenoic acid, a major component of fish oils responsible for augmentation of endothelial function. Fifth, if endothelial dysfunction plays a primary role in pathogenesis of coronary vasospasm, then the prevalence and severity of endothelial dysfunction in the coronary artery would be greater and more frequent in Asian patients with vasospastic angina than in their Caucasian counterparts; however, this may not be the case. Finally, contractility of coronary smooth muscle cells is indeed augmented at the spastic coronary segment in a patient with

vasospastic angina and in porcine models of coronary artery spasm.

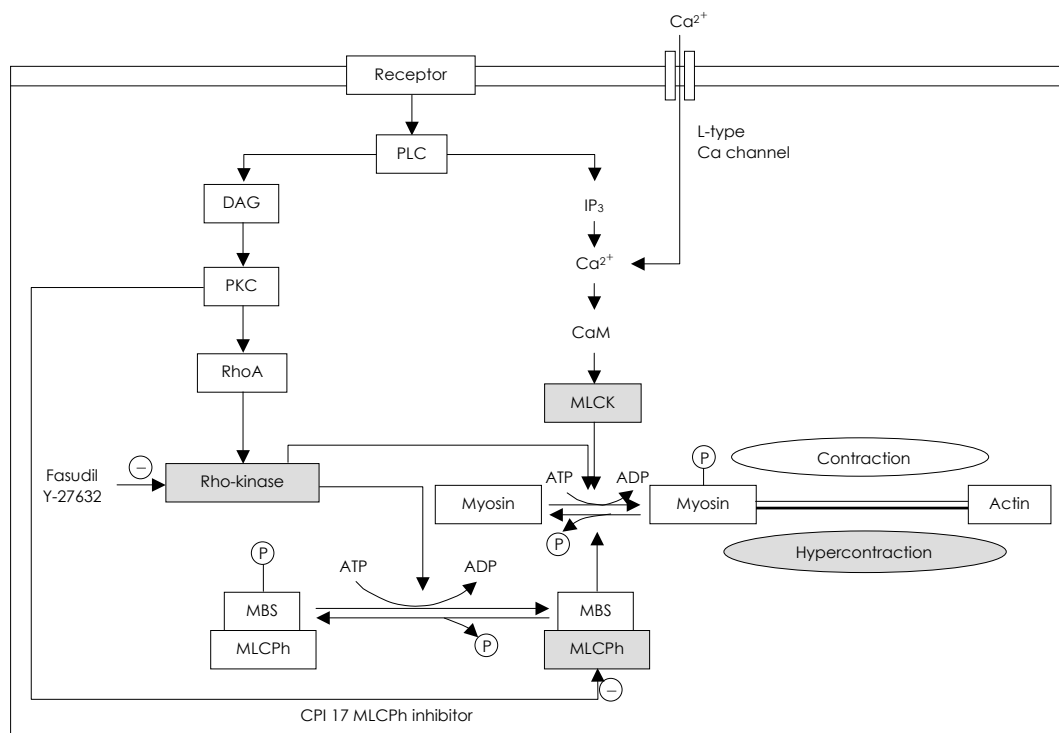
### Enhanced Vascular Smooth Muscle Contractility

Vascular smooth muscle contraction and relaxation are regulated by phosphorylation and dephosphorylation of myosin light chain by myosin light chain kinase (MLCK) and myosin light chain phosphatase (MLCP).<sup>59)</sup> Vascular smooth muscle contraction is initiated by binding of various agonists or stimuli, such as serotonin or histamine, to their receptors; phospholipase C is activated, leading to formation of inositol 1,4,5-triphosphate (IP<sub>3</sub>) and diacylglycerol (DAG). IP<sub>3</sub> then binds to its receptor on the membrane of the sarcoplasmic reticulum (SR) to mobilize stored calcium ions (Ca<sup>2+</sup>) into the cytosol. The Ca<sup>2+</sup>/calmodulin complex then activates MLCK, with subsequent phosphorylation of myosin light chain.<sup>60)</sup> Thus, the more intracellular Ca<sup>2+</sup> concentration is increased, the more contraction of vascular smooth muscle will occur. This is known as a classical pathway for vascular smooth muscle contraction.

However, it is also well known that the relationship between elevation of cytosolic Ca<sup>2+</sup> concentration and the degree of developed tension varies depending on the

situation.<sup>61)</sup> The Ca<sup>2+</sup>-tension relationship changes during the time course of the contraction, in which the sustained phase of the contraction is maintained by a relatively lower level of cytosolic Ca<sup>2+</sup>. This phenomenon is referred to as "Ca<sup>2+</sup> sensitization of the contractile apparatus" or "an increase in Ca<sup>2+</sup> sensitivity".<sup>59)</sup> Therefore, contraction of vascular smooth muscle is subjected to dual regulation by the Ca<sup>2+</sup>-dependent and the so-called Ca<sup>2+</sup> independent signal. Ca<sup>2+</sup> sensitivity can be regulated by either myosin light chain phosphorylation-dependent or -independent mechanisms, while phosphorylation-dependent mechanisms are considered to play a major role in regulation of Ca<sup>2+</sup> sensitivity.<sup>62)</sup> However, investigation of the molecular mechanisms of enhanced smooth muscle responsiveness in patient with vasospastic angina is not easy, due to the difficulty of obtaining tissue specimens of human coronary spastic segments.

Recent evidence indicates that small GTPase RhoA and its downstream effector, ROCK/Rho-kinase, inhibits MLCP, leading to augmentation of myosin light chain phosphorylation and Ca<sup>2+</sup> sensitization in response to vasoconstrictor stimuli (Fig. 3). In order to elucidate the molecular mechanism of coronary artery spasm, Shimokawa and colleagues<sup>58)</sup> have developed porcine models of coronary artery spasm by chronic application of interleukin- $\beta$  (IL- $\beta$ ) to the coronary artery of porcine and by re-



**Fig. 3.** Molecular mechanisms of coronary artery spasm. Upon stimulation by various agonists, the Rho/Rho-kinase-mediated pathway is activated, resulting in inhibition of myosin phosphatase (via phosphorylation of its myosin binding subunit), with a resultant increase in myosin light-chain phosphorylation and vascular smooth muscle hypercontraction. By contrast, the contribution of intracellular Ca<sup>2+</sup> release may be minimal. With regard to the Rho/Rho-kinase-mediated pathway, several alterations could be involved, including enhanced expression of Rho/Rho-kinase, increased Rho-kinase activity, and inhibition of myosin phosphatase activity, all of which could eventually enhance myosin light-chain phosphorylation. PLC: phospholipase C, DAG: diacylglycerol, PKC: protein kinase C, IP<sub>3</sub>: inositol-1,4,5-triphosphate, CaM: calmodulin, MLC: myosin light chain, MBS: myosin binding subunit, MLCK: myosin light-chain kinase (Adapted and modified from reference 68).

removal of the endothelium with high-cholesterol feeding. Significantly increased and upregulated expression of ROCK/Rhokinase messenger RNA (mRNA) at the spastic rather than the control segment was demonstrated in this porcine model. They also showed that Fasudil, a specific inhibitor of ROCK/Rhokinase, inhibited coronary artery spasms in animals<sup>63</sup> and humans.<sup>64</sup> These results indicate that ROCK/Rhokinase plays a key role in induction of vascular smooth muscle hypercontraction by inhibition of MLCP through phosphorylation of myosin binding subunit.<sup>59</sup> The exact mechanisms by which the activity of ROCK/Rho-kinase is increased remain to be elucidated.

Recent studies have shown that eNOS is regulated by the ROCK/Rho-kinase pathway. Inhibition of RhoA geranylgeranylation by statins decreases membrane GTP-bound active RhoA and subsequent ROCK activity, leading to upregulation and activation of eNOS;<sup>65</sup> furthermore, direct inhibition of the ROCK/Rho-kinase pathway has been shown to increase eNOS expression.<sup>66</sup> Thus, ROCK/Rho-kinase is closely associated with endothelial NO activity.

## Conclusion

Although coronary artery spasm plays an important role in pathogenesis of many types of ischemic heart disease, the cellular and molecular mechanisms of the spasm are still not well understood. It is possible that patients with vasospastic angina might have a disturbance in endothelial function, as well as an enhanced response of the vascular smooth muscle of the coronary artery and some final common molecular mechanisms. Further studies are required for clarification of the molecular and cellular mechanisms of this little-known disease of the coronary artery.

## REFERENCES

- 1) Sueda S, Kohno H, Fukuda H, Uraoka T. *Did the widespread use of long-acting calcium antagonists decrease the occurrence of variant angina?* *Chest* 2003;124:2074-8.
- 2) Yoo SY, Shin DH, Jeong JI, et al. *Long-term prognosis and clinical characteristics of patients with variant angina.* *Korean Circ J* 2008;38:651-8.
- 3) Curry RC, Pepine CJ, Sabom MB, Feldman RL, Christie LG, Conti CR. *Effects of ergonovine in patients with and without coronary artery disease.* *Circulation* 1977;56:803-9.
- 4) Maseri A, Davies G, Hackett D, Kaski JC. *Coronary artery spasm and vasoconstriction: the case for a distinction.* *Circulation* 1990;81:1983-91.
- 5) Barbara AK, Daniel S, Andrew IS. *Hemostasis, thrombosis, fibrinolysis, and cardiovascular disease.* In: Libby P, Braunwald E editors. *Braunwald's Heart disease: A Textbook of Cardiovascular Medicine.* 8th ed. Philadelphia: Saunders company; 2008. p.2049.
- 6) Verma S, Anderson TJ. *Fundamentals of endothelial function for the clinical cardiologist.* *Circulation* 2002;105:546-9.
- 7) Rubanyi GM. *The role of endothelium in cardiovascular homeostasis and diseases.* *J Cardiovasc Pharmacol* 1993;22 (Suppl 4): S1-14.
- 8) Rafii S, Lyden D. *Therapeutic stem and progenitor cell transplantation for organ vascularization and regeneration.* *Nat Med* 2003;9:702-12.
- 9) Gulati R, Jevremovic D, Peterson TE, et al. *Autologous culture-modified mono-nuclear cells confer vascular protection after arterial injury.* *Circulation* 2003;108:1520-6.
- 10) Aicher A, Heecheen C, Mildner-Rihm C, et al. *Essential role of endothelial nitric oxide synthase for mobilization of stem and progenitor cells.* *Nat Med* 2003;9:1370-6.
- 11) Moncada S, Higgs A. *The L-arginine-nitric oxide pathway.* *N Engl J Med* 1993;329:2002-12.
- 12) Moncada S, Palmer RM, Higgs EA. *Nitric oxide: physiology, pathophysiology, and pharmacology.* *Pharmacol Rev* 1991;43:109-42.
- 13) Ignarro LJ. *Nitric oxide as a unique signaling molecule in the vascular system: a historical overview.* *J Physiol Pharmacol* 2002;53:503-14.
- 14) Hill JM, Zalos G, Halcox JP, et al. *Circulating endothelial progenitor cells, vascular function, and cardiovascular risk.* *N Engl J Med* 2003;348:593-600.
- 15) Lerman A, Zeiher AM. *Endothelial function: cardiac events.* *Circulation* 2005;111:363-8.
- 16) Vanhoutte PM. *Endothelial control of vasomotor function: from health to coronary disease.* *Circ J* 2003;67:572-5.
- 17) Kawashima S, Yokoyama M. *Dysfunction of endothelial nitric oxide synthase and atherosclerosis.* *Arterioscler Thromb Vasc Biol* 2004;24:998-1005.
- 18) Maseri A, L'Abbate A, Biagini A, et al. *Coronary vasospasm as a possible cause of myocardial infarction: a conclusion derived from the study of "preinfarction" angina.* *N Engl J Med* 1978;299:1271-7.
- 19) MacAlpin RN. *Angiographic determination of disease-produced alterations of vasomotor tone in large coronary arteries.* *Can J Cardiol* 1986; (Suppl A):209A-18A.
- 20) Maseri A, Severi S, Nes MD, et al. *"Variant" angina: one aspect of a continuous spectrum of vasospastic myocardial ischemia: pathogenic mechanisms, estimated incidence and clinical and coronary arteriographic findings in 138 patients.* *Am J Cardiol* 1978;42:1019-35.
- 21) MacAlpin RN. *Correlation of the location of coronary arterial spasm with the lead distribution of ST segment elevation during variant angina.* *Am Heart J* 1980;99:555-64.
- 22) Miyao Y, Kugiyama K, Yawano H, et al. *Diffuse intimal thickening of coronary arteries in patients with coronary spastic angina.* *J Am Coll Cardiol* 2000;36:432-7.
- 23) Suzuki H, Kawai S, Aizawa T, et al. *Histological evaluation of coronary plaques in patients with variant angina: relationship between vasospasm and neointimal hyperplasia in primary coronary lesions.* *J Am Coll Cardiol* 1999;33:198-205.
- 24) Shimokawa H, Tomioke H, Nabeyama S, et al. *Coronary artery spasm induced in atherosclerotic miniature swine.* *Science* 1983;221:560-2.
- 25) Shimokawa H, Tomioke H, Nabeyama S, et al. *Coronary artery spasm induced in miniature swine: angiographic evidence and relation to coronary atherosclerosis.* *Am Heart J* 1985;110:300-10.
- 26) Miwa K, Fujita M, Sasayama S. *Recent insights into the mechanisms, predisposing factors, and racial differences of coronary vasospasm.* *Heart Vessels* 2005;20:1-7.
- 27) Suzuki Y, Tokunaga S, Ikegushi S, et al. *Induction of coronary artery spasm by intracoronary acetylcholine: comparison with intracoronary ergonovine.* *Am Heart J* 1992;124:39-47.
- 28) Kanazawa K, Suematsu M, Ishida T, et al. *Disparity between serotonin- and acetylcholine-provoked coronary artery spasm.* *Clin*

- Cardiol* 1997;20:146-52.
- 29) Egashira K, Katsuda Y, Mohri M, et al. Basal release of endothelium-derived nitric oxide at site of spasm in patients with variant angina. *J Am Coll Cardiol* 1996;27:1444-9.
  - 30) Yamamoto H, Yoshimura, Noma M, et al. Preservation of endothelium-dependent vasodilatation in the spastic segment of the human epicardial coronary artery by substance P. *Am Heart J* 1992;123:298-303.
  - 31) Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980;288:373-6.
  - 32) Okumura K, Yasue H, Horio Y, et al. Multi-vessel coronary spasm in patients with variant angina: a study with intracoronary injection of acetylcholine. *Circulation* 1988;77:535-42.
  - 33) Kugiyama K, Yasue H, Okumura K, et al. Nitric oxide activity is deficient in spasm arteries of patients with coronary spastic angina. *Circulation* 1996;94:266-71.
  - 34) Matsuyama K, Yasue H, Okumura K, et al. Effects of H1-receptor stimulation on coronary arterial diameter and coronary hemodynamics in human. *Circulation* 1990;81:65-71.
  - 35) Bassenge E. Endothelium-mediated regulation of coronary tone. *Basic Res Cardiol* 1991;86 (Suppl 2):69-76.
  - 36) Yoshimura M, Yasue H, Nakayama M, et al. A missense Glu298-Asp variant in the endothelial nitric oxide synthase gene is associated with coronary spasm in the Japanese. *Hum Genet* 1998;103:65-9.
  - 37) Nakayama M, Yasue H, Yoshimura M, et al. T<sup>786</sup> → C mutation in the 5'-flanking region of the endothelial nitric oxide synthase gene is associated with coronary spasm. *Circulation* 1999;99:2864-70.
  - 38) Sugiishi M, Takatsu F. Cigarette smoking is a major risk factor for coronary spasm. *Circulation* 1993;87:76-9.
  - 39) Murohara T, Kugiyama K, Ohgushi M, Sugiyama S, Yasue H. Cigarette smoke extract contracts isolated porcine coronary arteries by superoxide anion-mediated degradation of EDRF. *Am J Physiol* 1994;266:H874-80.
  - 40) Ota Y, Kugiyama K, Sugiyama S, et al. Impairment of endothelium-dependent relaxation of rabbit aortas by cigarette smoke extract: role of free radicals and attenuation by captopril. *Atherosclerosis* 1997;131:195-202.
  - 41) Sugiyama S, Kugiyama K, Ohgushi M, et al. Supersensitivity of atherosclerotic artery to constrictor effect of cigarette smoke extract. *Cardiovasc Res* 1998;38:508-15.
  - 42) Kugiyama K, Motoyama T, Hirashima O, et al. Vitamin C attenuates abnormal vasomotor reactivity in spasm coronary arteries in patients with coronary spastic angina. *J Am Coll Cardiol* 1998;32:103-9.
  - 43) Motoyama T, Kawano H, Kugiyama K, et al. Vitamin E administration improves impairment of endothelium-dependent vasodilation in patients with coronary spastic angina. *J Am Coll Cardiol* 1998;32:1672-9.
  - 44) Mackness B, Durrington PN, Mackness MI. Polymorphisms of paraoxonase genes and low-density lipoprotein lipid peroxidation. *Lancet* 1999;353:468-9.
  - 45) Itoh T, Yasue H, Yoshimura M, et al. Paraoxonase gene Gln192-Arg (Q192R) polymorphism is associated with coronary artery spasm. *Hum Genet* 2002;110:89-94.
  - 46) Shepherd JT, Katsis ZS. Endothelium derived vasoactive factors: I. endothelium-dependent relaxation. *Hypertension* 1991;18 (5 Suppl):III76-85.
  - 47) Boulanger C, Luscher TF. Release of endothelin from the porcine aorta: inhibition by endothelium-derived nitric oxide. *J Clin Invest* 1990;85:587-90.
  - 48) Takemoto M, Egashira K, Usui M, et al. Important role of tissue angiotensin converting enzyme activity in the pathogenesis of coronary vascular and myocardial structural changes induced by long-term blockade of nitric oxide synthesis in rats. *J Clin Invest* 1997;99:278-87.
  - 49) Moncada S, Vane JR. Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A2 and prostacyclin. *Pharmacol Rev* 1978;30:293-331.
  - 50) Feletou M, Vanhoutte PM. Endothelium-dependent hyperpolarization of canine coronary smooth muscle. *Br J Pharmacol* 1988;93:515-24.
  - 51) Yanagisawa M, Kurihara H, Kimura S, et al. A novel vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 1988;332:411-5.
  - 52) Luscher TF, Vanhoutte PM. Endothelium-dependent contractions to acetylcholine in the aorta of the spontaneously hypertensive rat. *Hypertension* 1986;8:344-8.
  - 53) Lewis JR, Kisilevsky R, Armstrong PW. Prinzmetal's angina, normal coronary artery and pericarditis. *Can Med Assoc J* 1978;119:36-9.
  - 54) Li JJ, Nie SP, Xu B, Guo YL, Gao Z, Zheng X. Inflammation in variant angina: Is there any evidence? *Med Hypotheses* 2007;68:635-40.
  - 55) Kaikita K, Ogawa H, Yasue H, et al. Soluble P-selectin is released into the coronary circulation after coronary spasm. *Circulation* 1995;92:1726-30.
  - 56) Miwa K, Igawa A, Inoue H. Soluble E-selectin, ICAM-1 and VCAM-1 levels in systemic and coronary circulation in patients with variant angina. *Cardiovasc Res* 1997;36:37-44.
  - 57) Hung MJ, Cherng WJ, Yang NI, Cheng CW, Li LF. Relation of high-sensitivity C-reactive protein level with coronary vasospastic angina pectoris in patients without hemodynamically significant coronary artery disease. *Am J Cardiol* 2005;96:1484-90.
  - 58) Shimokawa H. Cellular and molecular mechanisms of coronary artery spasm-lessons from animal models. *Jpn Circ J* 2000;64:1-12.
  - 59) Somlyo AP, Somlyo AV. Signal transduction and regulation in smooth muscle. *Nature* 1994;372:231-6.
  - 60) Kimura K, Ito M, Amano M, et al. Regulation of myosin phosphatase by Rho and Rho-associated kinase (Rho kinase). *Science* 1996;273:245-8.
  - 61) Hirano K, Hirano M, Abe S, et al. Ion channels of vascular smooth muscle cells and endothelial cells. *New-York: Elsevier; 1991. p. 93-105.*
  - 62) Hirano K. Current topics in the regulatory mechanism underlying the Ca<sup>2+</sup> sensitization of the contractile apparatus in vascular smooth muscle. *J Pharmacol Sci* 2007;104:109-15.
  - 63) Kandabashi T, Shinokawa H, Miyata K, et al. Inhibition of myosin phosphatase by upregulated Rho-kinase plays a key role for coronary artery spasm in a porcine model with interleukin-1 $\beta$ . *Circulation* 2000;101:1319-23.
  - 64) Masumoto A, Mohri M, Shimokawa H, Urakami L, Usui M, Takeshita A. Suppression of coronary artery spasm by the Rho-kinase inhibitor fasudil in patients with vasospastic angina. *Circulation* 2002;105:1545-7.
  - 65) Laufs U, Liao JK. Post-transcriptional regulation of endothelial nitric oxide synthase mRNA stability by Rho GTPase. *J Biol Chem* 1998;273:24266-71.
  - 66) Wolfrum S, Dendorfer A, Rikitake Y, et al. Inhibition of Rho-kinase leads to rapid activation of phosphatidylinositol 3-kinase/protein kinase Akt and cardiovascular protection. *Arterioscler Thromb Vasc Biol* 2004;24:1842-7.
  - 67) Murad F. Nitric oxide and cyclic GMP in cell signaling and drug development. *N Engl J Med* 2006;355:2003-11.
  - 68) Shimokawa H. Rho-kinase as a novel therapeutic target in treatment of cardiovascular disease. *J Cardiovasc Pharmacol* 2002;39:319-27.