DDR

DRUG DEVELOPMENT RESEARCH 60:14–19 (2003)

Research Overview

Searching for the Physiological Role and Therapeutic Potential of Vascular Proteinase-Activated Receptor-2 (PAR_2)

John J. McGuire* and Chris R. Triggle

Smooth Muscle Research Group and the Canadian Institutes of Health Research Group on the Regulation of Vascular Contractility, Department of Pharmacology & Therapeutics, Faculty of Medicine, University of Calgary, Calgary, Alberta Canada

Strategy, Management and Health Policy				
Venture Capital Enabling Technology	Preclinical Research	Preclinical Development Toxicology, Formulation Drug Delivery, Pharmacokinetics	Clinical Development Phases I-III Regulatory, Quality, Manufacturing	Postmarketing Phase IV

ABSTRACT The intercellular interactions of endothelial and vascular smooth muscle cells are interesting to many scientists who seek to develop new drugs to treat cardiovascular diseases. Of particular interest is the regulation of blood flow by the paracrine actions of the endothelium on the underlying vascular smooth muscle cells of blood vessels. The development and further understanding of drugs that mimic or potentiate endothelial-derived factors, in particular vasodilators such as nitric oxide (NO), have proved to be of therapeutic benefit (e.g., sildenafil, nitroglycerin). On the other hand, endothelial-derived proinflammatory substances are released in response to tissue insult or during the progression of vascular diseases such as atherosclerosis. Proteinase-activated receptor 2 (PAR_2) represents a novel target for vascular biology because of 1) its unique mechanism of activation by proteinases, 2) questions regarding the identity of the endogenous agonist(s), and 3) its apparent multiple activities in the vasculature. Whether it will be agonists or antagonists of PAR_2 that will serve as the basis for a new class of therapeutic agents for the treatment of vascular diseases is an open question for further research and drug development. Drug Dev. Res. 60:14–19, 2003.

Key words: endothelium; PAR_2; EDHF; nitric oxide; vascular smooth muscle

CURRENT LINES OF RESEARCH AND SCOPE OF ARTICLE

Among the earliest experimental observations in vitro reported regarding the activation of proteinase-activated receptor 2 (PAR_2) either by enzyme or peptide agonists was the endothelium-dependent vasodilation of isolated blood vessels [Hollenberg et al., 1996; Al Ani et al., 1995]. The complete inhibition of vasodilation by an inhibitor of nitric oxide (NO) synthases made it clear that NO mediated this response to PAR_2 activation in many blood vessels [Hollenberg et al., 1996; Moffatt and Cocks, 1998; Sobey and Cocks, 1998; Glusa et al., 1997; Magazine et al., 1996]. However, it was observed subsequently that NO produced by the endothelium did not entirely account for the vasodilation or the blood pressure lowering effects that resulted from PAR_2 activation, nor did products of cyclooxygenases [Damiano et al., 1999a; Emilsson et al., 1997]. Thus, it was proposed that unidentified endothelium-dependent hyperpolarization factor(s) (EDHFs) were also involved [Trottier et al.,

Contract grant sponsors: Heart and Stroke Foundation of Canada; Heart and Stroke Foundation of Alberta, Northwest Territories and Nunavut; Canadian Institutes for Health Research.

*Correspondence to: John J. McGuire, PhD, Smooth Muscle Group, University of Calgary, Calgary, Alberta T2N 4N1, Canada. E-mail: mcguire@ucalgary.ca

Published online in Wiley InterScience (www.interscience.wiley.com) DOI: 10.1002/ddr.10314

2002; McGuire et al., 2002a; McLean et al., 2002; Hamilton and Cocks, 2000]. In addition to affecting the tone of vascular smooth muscle indirectly through the endothelium, the direct activation of PAR_2 expressed on the smooth muscle cells resulted in vasoconstriction of some blood vessels [Magazine et al., 1996; Moffatt and Cocks, 1998]. Therefore, the expression of PAR_2 on both endothelial and vascular smooth muscle influences vascular reactivity.

Paradoxically, in experimental models of both chronic hypertension (spontaneously hypertensive rats [Sobey et al., 1999]) and hypotension (sepsis [Cicala et al., 1999]), the expression of PAR_2 was upregulated in vivo. Interestingly, PAR_2 expression was also increased during arterial restenosis after balloon angioplasty in yet another animal model [Damiano et al., 1999b]. Furthermore, the PAR_2-mediated vasodilation of coronary arteries was selectively preserved compared with the reduced impact of other endothelial G protein-coupled receptors in a model of global cardiac ischemia–reperfusion injury [McLean et al., 2002]. This study by McLean et al. [2002] indicates that the PAR_2-mediated release of an EDHF, pharmacologically identified as a lipoxygenase-derived eicosanoid, is preserved after ischemia–reperfusion injury, and it is likely that this PAR_2-regulated pathway plays a protective role in this situation. Thus, PAR_2 expression increased after a variety of pathophysiological stresses on the vasculature. These observations are consistent with cell culture experiments wherein various cytokines and proinflammatory substances induced changes in the cellular expression of PAR_2 in endothelial and vascular smooth muscle cells [Nystedt et al., 1996; Bono et al., 1997]. PAR_2 modulates, either directly or indirectly, vascular tone and blood flow, but for what purpose? And to what end? The admittedly speculative drawing below reflects our current thoughts on PAR_2 regulation of vascular tone (Fig. 1).

The scope of this review article is to highlight several lines of research involving PAR_2 and the vasculature. The main aim is to present the information in a context of proposals for the future development of drugs to treat cardiovascular diseases, based on the acute pharmacological effects that activation of PAR_2 can exert on vascular tone.

ENDOGENOUS AGONISTS OF VASCULAR PAR_2: PHARMACOLOGY, OR PHYSIOLOGY?

Distinguishing the importance of the pharmacological actions of PAR_2 from its putative physiological roles in the vasculature depends partially on characterizing its endogenous activators that are presumably site-generated serine proteinases. This distinction between the physiological roles and pharmacological activities for PAR_2 is difficult because PAR_2 antagonists have yet to be discovered. Given the irreversible nature of PAR_2 activation, in which the tethered ligand remains attached to the receptor, presents a situation wherein physiological regulation of the activation of PARs requires tight control of agonist enzyme activities and of the receptor desensitization processes. Trypsin is a candidate for PAR_2 activation in the gastrointestinal system; but is trypsin a plausible agonist for PAR_2 in the vasculature? This may exist, because mRNA encoding trypsinogens 1 and 2, the proenzymes of trypsin 1 and 2, respectively, were detected in human umbilical vein endothelial cells in cell culture [Koshikawa et al., 1997]. It is tempting to speculate that endothelial-derived trypsin activates PAR_2 in vivo either in an autocrine manner or in a paracrine manner by activating PAR_2 on surrounding cells. Trypsin is unlikely to be the sole mediator of vascular reactivity via PAR_2. For instance, the milieu of blood and interstitial media that surround the vascular smooth muscle and endothelial cells contains endogenous inhibitors of trypsin (so-called serpins), and thus, the effects of PAR_2 activation by trypsin in the vasculature might only be observed under conditions of protease inhibitor imbalance.

Alveolar angiogenesis, a key event in tumor progression in the lung, is proposed to involve PAR_2 [Jin et al., 2003], providing yet another venue for the impact of PARs on vascular physiology. Tumorigenic cells produce a variety of proteinases that could potentially regulate PARs. Thus, the therapeutic potential of inhibitors of serine proteinases as cancer chemotherapeutic agents may be due in part to their impact on PAR activation [Kennedy, 1998; Meyskens, 2001]. It can be noted further that PAR_2 agonists stimulate protective angiogenesis in a model of hindlimb ischemia [Milia et al., 2002]. Therefore, one can speculate that PAR_2 plays a role in the vasculature not only via the acute effects on vascular tone, but also in the setting of either normal or pathological angiogenesis.

Human mast cell tryptase has a selective specificity for activating the PAR_2 compared with the other PARs. Unlike trypsin, to date no endogenous inhibitors of tryptase activity have been discovered. However, posttranslational modification of vascular PAR_2 itself modulates the responsiveness of PAR_2 to this enzyme in the vasculature [Compton et al., 2002]. Although further studies are ongoing, it is worth speculating that differential glycosylation of PAR_2 on endothelial and vascular smooth cells or a disruption of the normal glycosylation of PAR_2 may determine its responsiveness to a variety of proteinases such as tryptase in different vascular beds. Tryptase is a biomarker for human mast

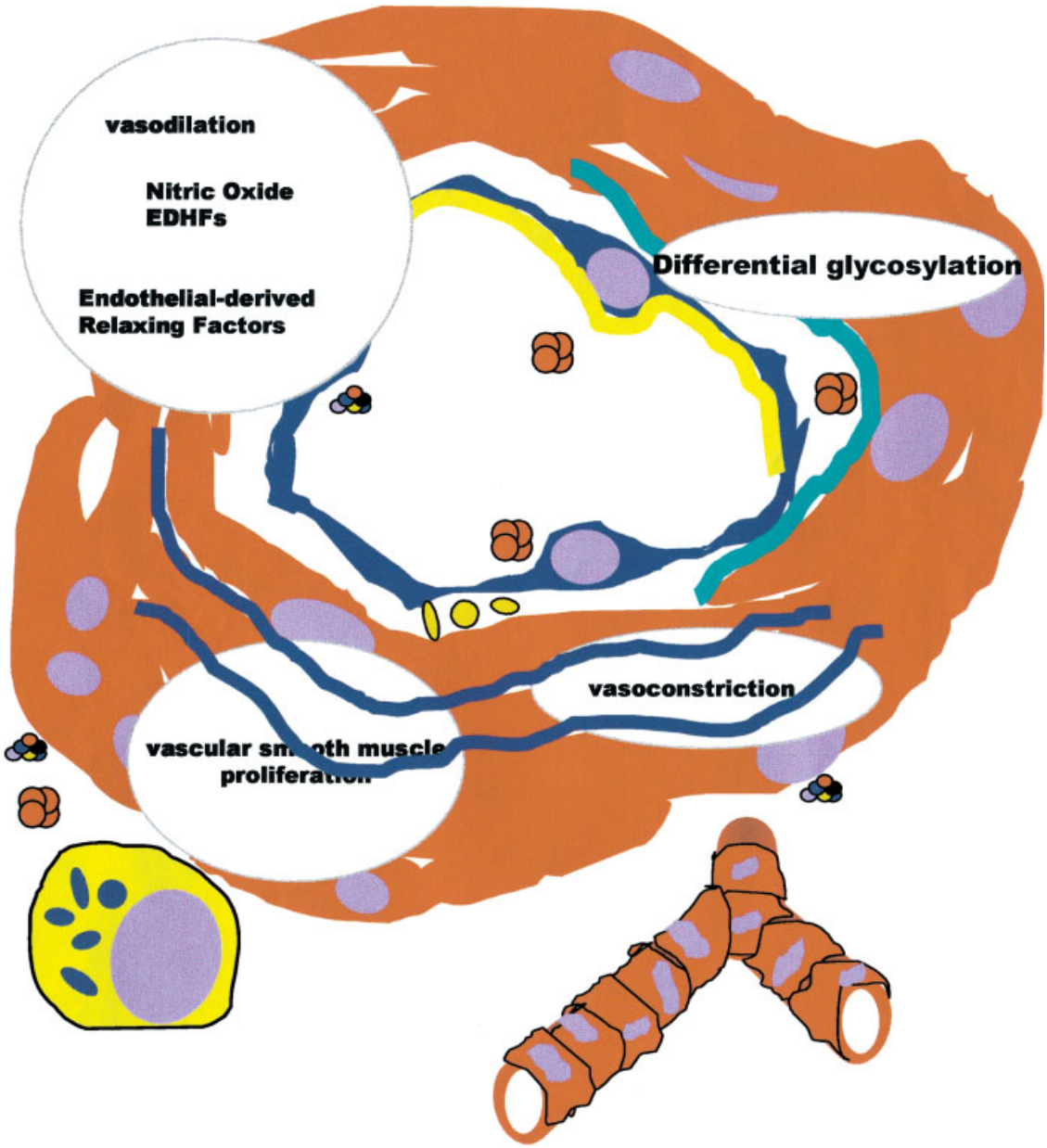

Fig. 1. Effects of proteinase-activated receptor 2 (PAR_2) on blood vessels. Clockwise from top left: The activation of PAR_2 on endothelial cells by either peptides or specific enzymes such as mast cell tryptase produces vasodilator factors that act on the underlying vascular smooth muscle. Differential glycosylation of PAR_2 on different cell types, in different vascular beds or in disease states, may regulate the activation of PAR_2 by enzymes such as tryptase that, at least to date, have no known endogenous inhibitors. When the vasodilator effects of PAR_2 are blocked with inhibitors or are reduced by endothelial dysfunction, the activation of PAR_2 on vascular smooth muscle cells may cause vasoconstriction. Extended duration of PAR_2 activation is associated with vascular smooth muscle proliferation as well as angiogenesis. Activation of endothelial PAR_2 induces the production of inflammatory substances such as interleukin-6. Cytokines induce an upregulation of PAR_2 expression on vascular smooth muscle cells. This upregulation may be associated with the smooth muscle cell proliferation that occurs in atherosclerosis. Furthermore, cells like mast cells involved in innate defense mechanisms may contribute to the progression of vascular diseases through the activation and upregulation of PAR_2 via the release of PAR_2 agonist, proteinases, or receptor-inducing cytokines, respectively. EDHFs, endothelium-dependent hyperpolarization factors.

cell activation and is present at sites of tissue injury, including the outer layers of blood vessels during the development of vascular atherosclerotic lesions [Kovanen et al., 1995]. Dysfunctional endothelium is present in such blood vessels, and endothelial dysfunction is an early indicator of cardiovascular disease [Pannirselvam et al., 2003]. Therefore, despite the expression of PAR_2 on the endothelium due to

endothelial dysfunction, the production of endothelium-derived factors such as NO, via PAR_2 activation, would be lost. Furthermore, changes in PAR_2 expression on vascular smooth muscle cells might increase the receptor distribution, resulting in a greater activation of PAR_2 on these cells. This increased activation could explain the reported vasoconstriction of some blood vessels and the hyperplasia of rat aortic smooth muscle cells in the setting of vascular injury [McGuire et al., 2002b; Moffatt and Cocks, 1998; Bono et al., 1997]. New therapeutic agents to target posttranslational glycosylation of PAR_2 or drugs that could inhibit selectively mast cell tryptase, i.e., without inhibiting other serine proteinases, might therefore be useful for treatment of cardiovascular diseases that involve a vascular inflammatory (e.g., atherosclerosis) or a proliferative (e.g., tumor angiogenesis, postangioplasty restenosis) component.

A number of enzymes involved in blood coagulation and hemostasis mediate their effects either entirely or partly via the activation of the PARs (see Riewald and Ruf, this Special Issue of Drug Development Research 59(4):400). The zymogen, Factor X, is regulated tightly by its conversion to the active form (Factor Xa) and requires formation of multiprotein complexes with other blood-clotting factors (e.g., Factor VIIa). Factor Xa displays greater selectivity for the activation of PAR_2 than for other PARs [Camerer et al., 2002], albeit with a lower specific activity relative to other enzyme substrates. PAR_2 is distinct from PAR_1, PAR_3, and PAR_4 because of its agonist enzyme specificity. Unlike PARs 1, 3, and 4, PAR_2 is not targeted by the blood-clotting enzyme, thrombin. However, it is anticipated that the function of vascular PAR_2 is coordinated, via a mechanism yet to be determined, with the functions of the other PARs. The hemodynamic responses to PAR_2 agonists were unchanged in PAR_1(-/-) mice; but agonists of PAR_1 produced greater responses in PAR_2(-/-) mice, thus suggesting an upregulation of PAR_1 in the setting of a deficiency of PAR_2. The upregulation of PARs in cardiovascular pathophysiology is discussed in more detail by Cirino and Cicala (see the article in this issue of DDR).

ENDOTHELIAL AND VASCULAR SMOOTH MUSCLE CELL PAR_2 AS TARGETS FOR DRUG DEVELOPMENT: AGONISTS? OR ANTAGONISTS?

Simply put, the physiological role of endothelial PAR_2 is not known. Is it present as a silent receptor awaiting its activation in disease or inflammation? Or is it actively participating in the regulation of blood flow and vascular tone? Because the transgenic PAR_2-deficient mice maintain a normal blood pressure, it would appear that rather than acting as a homeostatic regulator of vascular hemodynamics, it is more likely that that PAR_2 plays a role in vascular disease or inflammatory states [Damiano et al., 1999a]. However, the interpretation of data obtained from receptor-deficient mice should be done with caution because redundancy in cellular signaling processes is common. For instance, in the endothelial nitric oxide synthase knockout mouse (eNOS -/-), the contribution of EDHF is upregulated, at least in the resistance vasculature [Waldron et al., 1999; Ding et al., 2000]. The upregulation of EDHF in the eNOS -/- mouse may explain why this mouse reflects only a modest elevation of blood pressure [Huang et al., 1995]. Given a "normal" vascular phenotype in the PAR_2-deficient mouse, PAR_2 antagonists would not be expected to have undesirable effects on vascular function and hemodynamics. However, more in-depth studies are needed to evaluate the consequences of unbalancing the hemodynamics in these PAR_2-deficient mice before it can be concluded that there is no homeostatic role for PAR_2. Importantly, it has yet to be determined whether the upregulation of PAR_2 may contribute either to pathogenesis or to a protective mechanism in the setting of disease (see Cirino and Cicala, this Special Issue of Drug Development Research 60(1):20). The acute activation of PAR_2 with selective peptide agonists results in vasodilation of isolated blood vessels in vitro and lowers blood pressure when administered in vivo [McGuire et al., 2002a; Damiano et al., 1999a]. Recently, the first in-human study, which utilized a PAR_2 activating peptide and then measured dorsal hand vein or arterial forearm arterial blood flow, reported that PAR_2 agonist treatment was well tolerated and no ill effects were detected [Robin et al., 2003]. An inhibitor of NO synthase or aspirin partially reduced the vasodilation by the PAR_2 agonist, but because the combination of inhibitors was not tested, a putative residual "EDHF" component to vasodilation was not excluded from the vasodilator response [Robin et al., 2003]. Therefore, different cardiovascular diseases might benefit from treatment with agonists of endothelial PAR_2 (congestive heart failure; ischemia–reperfusion; erectile dysfunction), whereas in other vascular crises, the antagonists might be useful (sepsis-induced hypotension).

Drugs such as nitroglycerin and sildenafil have proved of use for treatment of the symptoms of many vascular-related diseases. These agents work essentially by mimicking and potentiating the signal transduction mechanisms of endothelium-derived NO. Because activation of PAR_2 causes the release of endothelial nitric oxide and putative EDHF(s), agonists targeted selectively to vascular PAR_2 might be useful as adjunct therapies in combination with other vasoactive drugs.

Of course, the suitability of PAR_2 agonists or antagonists as drugs for the treatment of vascular diseases will depend on being able to distribute these compounds selectively to the appropriate vasculature. Thus, the broad receptor distribution of PAR_2 in many organs and tissues may make this approach difficult. However, the EDHF effect of activation of PAR_2 has been observed only in small resistance-like blood vessels and thus, the use of PAR_2 agonists could be used to alter vascular resistance and treat peripheral vascular diseases.

In a limited number of studies, the activation of PAR_2 caused vasoconstriction in vascular preparations. This response required the inhibition of the production of endothelial-derived relaxation factors [McGuire et al., 2002b; Moffatt and Cocks, 1998; Nystedt et al., 1995]. Thus, the vasodilator or vasoconstrictor activities of PAR_2 depend on the relative endothelial versus vascular smooth muscle distribution of receptors, and hence, on the "health status" of the endothelium. Endothelium-derived relaxation factors such as those produced by activation of PAR_2 (NO and the unidentified EDHF) are thought generally to be vasoprotective. The production of EDHFs is proposed to be a compensatory mechanism for the loss of production of NO. Endothelial dysfunction, i.e., the loss of production of endothelial-derived relaxation factors, is associated with the development of vascular complications in metabolic diseases such as diabetes. Although it is not expected that correction of endothelial dysfunction would also correct the metabolic abnormalities of diabetes, restoring endothelial function might change the progression of diabetic vascular dysfunction, with its associated tissue morbidity. The function of vascular PAR_2 in diabetes has yet to be determined, but is of interest for future studies.

CONCLUSIONS

The therapeutic potential of drugs that target PAR_2 for treating vascular diseases appears to be great. However, this direction is currently problematic because there are unanswered questions regarding the true physiological role of this receptor. Furthermore, the endogenous agonists, presumably proteinases that activate PAR_2 in the cardiovascular system, have yet to be unequivocally identified. Although clues to answer these questions have been obtained with the use of selective PAR_2-activating peptides and with PAR_2-deficient mice, the synthesis of a selective PAR_2 antagonist, like the one available for PAR_1, would appear to be an essential tool to developing PAR_2-targeted therapeutic agents.

ACKNOWLEDGMENTS

The authors acknowledge a Post-Doctoral Fellowship from AstraZeneca/Canadian Institutes for Health Research/Heart and Stroke Foundation of Canada (to J.J.M.).

REFERENCES

Al Ani B, Saifeddine M, Hollenberg MD. 1995. Detection of functional receptors for the proteinase-activated-receptor-2-activating polypeptide, SLIGRL-NH_2, in rat vascular and gastric smooth muscle. Can J Physiol Pharmacol 73:1203–1207.

Bono F, Lamarche I, Herbert JM. 1997. Induction of vascular smooth muscle cell growth by selective activation of the proteinase activated receptor-2 (PAR-2). Biochem Biophys Res Commun 241:762–764.

Camerer E, Kataoka H, Kahn M, Lease K, Coughlin SR. 2002. Genetic evidence that protease-activated receptors mediate factor Xa signaling in endothelial cells. J Biol Chem 277:16081–16087.

Cicala C, Pinto A, Bucci M, Sorrentino R, Walker B, Harriot P, Cruchley A, Kapas S, Howells GL, Cirino G. 1999. Protease-activated receptor-2 involvement in hypotension in normal and endotoxemic rats in vivo. Circulation 99:2590–2597.

Compton SJ, McGuire JJ, Saifeddine M, Hollenberg MD. 2002. Restricted ability of human mast cell tryptase to activate proteinase-activated receptor-2 in rat aorta. Can J Physiol Pharmacol 80:987–992.

Damiano BP, Cheung WM, Santulli RJ, Fung-Leung WP, Ngo K, Ye RD, Darrow AL, Derian CK, de Garavilla L, Andrade-Gordon P. 1999a. Cardiovascular responses mediated by protease-activated receptor-2 (PAR-2) and thrombin receptor (PAR-1) are distinguished in mice deficient in PAR-2 or PAR-1. J Pharmacol Exp Ther 288:671–678.

Damiano BP, D'Andrea MR, de Garavilla L, Cheung WM, Andrade-Gordon P. 1999b. Increased expression of protease activated receptor-2 (PAR-2) in balloon-injured rat carotid artery. Thromb Haemost 81:808–814.

Ding H, Kubes P, Triggle C. 2000. Potassium- and acetylcholine-induced vasorelaxation in mice lacking endothelial nitric oxide synthase. Br J Pharmacol 129:1194–1200.

Emilsson K, Wahlestedt C, Sun MK, Nystedt S, Owman C, Sundelin J. 1997. Vascular effects of proteinase-activated receptor 2 agonist peptide. J Vasc Res 34:267–272.

Glusa E, Saft A, Prasa D, Sturzebecher J. 1997. Trypsin- and SLIGRL-induced vascular relaxation and the inhibition by benzamidine derivatives. Thromb Haemost 78:1399–1403.

Hamilton JR, Cocks TM. 2000. Heterogeneous mechanisms of endothelium-dependent relaxation for thrombin and peptide activators of protease-activated receptor-1 in porcine isolated coronary artery. Br J Pharmacol 130:181–188.

Hollenberg MD, Saifeddine M, Al Ani B. 1996. Proteinase-activated receptor-2 in rat aorta: structural requirements for agonist activity of receptor-activating peptides. Mol Pharmacol 49:229–233.

Huang PL, Huang Z, Mashimo H, Bloch KD, Moskowitz MA, Bevan JA, Fishman MC. 1995. Hypertension in mice lacking the gene for endothelial nitric oxide synthase. Nature 377:239–242.

Jin E, Fujiwara M, Pan X, Ghazizadeh M, Ara S, Ohaki Y, Kajiwara K, Takemura T, Kawanami O. 2003. Protease-activated receptor

(PAR)-1 and PAR-2 participate in the cell growth of alveolar capillary endothelium in primary lung adenocarcinomas. Cancer 97:703–713.

Kennedy AR. 1998. Chemopreventive agents: protease inhibitors. Pharmacol Ther 78:167–209.

Koshikawa N, Nagashima Y, Miyagi Y, Mizushima H, Yanoma S, Yasumitsu H, Miyazaki K. 1997. Expression of trypsin in vascular endothelial cells. FEBS Lett 409:442–448.

Kovanen PT, Kaartinen M, Paavonen T. 1995. Infiltrates of activated mast cells at the site of coronary atheromatous erosion or rupture in myocardial infarction. Circulation 92:1084–1088.

Magazine HI, King JM, Srivastava KD. 1996. Protease activated receptors modulate aortic vascular tone. Int J Cardiol 53(Suppl): 75–80.

McGuire JJ, Hollenberg MD, Andrade-Gordon P, Triggle CR. 2002a. Multiple mechanisms of vascular smooth muscle relaxation by the activation of proteinase-activated receptor 2 in mouse mesenteric arterioles. Br J Pharmacol 135:155–169.

McGuire JJ, Dai J, Andrade-Gordon P, Triggle CR, Hollenberg MD. 2002b. Proteinase-activated receptor-2 (PAR2): vascular effects of a PAR2-derived activating peptide via a receptor different than PAR2. J Pharmacol Exp Ther 303:985–992.

McLean PG, Aston D, Sarkar D, Ahluwalia A. 2002. Protease-activated receptor-2 activation causes EDHF-like coronary vasodilation: selective preservation in ischemia/reperfusion injury: involvement of lipoxygenase products, VR1 receptors, and C-fibers. Circ Res 90:465–472.

Meyskens FL. 2001. Development of Bowman-Birk inhibitor for chemoprevention of oral head and neck cancer. Ann NY Acad Sci 952:116–123.

Milia AF, Salis MB, Stacca T, Pinna A, Madeddu P, Trevisani M, Geppetti P, Emanueli C. 2002. Protease-activated receptor-2 stimulates angiogenesis and accelerates hemodynamic recovery in a mouse model of hindlimb ischemia. Circ Res 91:346–352.

Moffatt JD, Cocks TM. 1998. Endothelium-dependent and -independent responses to protease-activated receptor-2 (PAR-2) activation in mouse isolated renal arteries. Br J Pharmacol 125:591–594.

Nystedt S, Emilsson K, Larsson AK, Strombeck B, Sundelin J. 1995. Molecular cloning and functional expression of the gene encoding the human proteinase-activated receptor 2. Eur J Biochem 232:84–89.

Nystedt S, Ramakrishnan V, Sundelin J. 1996. The proteinase-activated receptor 2 is induced by inflammatory mediators in human endothelial cells. Comparison with the thrombin receptor. J Biol Chem 271:14910–14915.

Pannirselvam M, Anderson T, Triggle CR. 2003. Endothelial cell dysfunction in type I and II diabetes: the cellular basis for dysfunction. Drug Dev Res 58:28–41.

Robin J, Kharbanda R, Mclean P, Campbell R, Vallance P. 2003. Proteinase-activated receptor 2-mediated vasodilatation in humans in vivo—role of nitric oxide and prostanoids. Circulation 107:954–959.

Sobey CG, Cocks TM. 1998. Activation of protease-activated receptor-2 (PAR-2) elicits nitric oxide-dependent dilatation of the basilar artery in vivo. Stroke 29:1439–1444.

Sobey CG, Moffatt JD, Cocks TM. 1999. Evidence for selective effects of chronic hypertension on cerebral artery vasodilatation to protease-activated receptor-2 activation. Stroke 30: 1933–1940.

Trottier G, Hollenberg M, Wang X, Gui Y, Loutzenhiser K, Loutzenhiser R. 2002. PAR-2 elicits afferent arteriolar vasodilation by NO-dependent and NO-independent actions. Am J Physiol Renal Physiol 282:F891–F897.

Waldron GJ, Ding H, Lovren F, Kubes P, Triggle CR. 1999. Acetylcholine-induced relaxation of peripheral arteries isolated from mice lacking endothelial nitric oxide synthase. Br J Pharmacol 128:653–658.