PAR2 Is Partout and Now in the Heart

Andrew Maree, Desmond Fitzgerald

Endothelial cell injury is a common accompaniment of cardiovascular disease, often evident as an attenuation of endothelial-dependent vasorelaxation. Vascular generation of nitric oxide (NO) in response to a variety of agonists is impaired in diabetes, atherosclerosis, and even isolated hyperlipidemia. Altered NO production may contribute not only to abnormal vascular resistance but also to the development of smooth muscle cell proliferation within the vessel. An acute endothelial injury is sustained during reperfusion of ischemic myocardium. Free radical generation results in endothelial dysfunction, limiting coronary flow in the reperfused segment and further hindering myocardial function. Cardioprotective effects of NO are inhibited; indeed, its interaction with superoxide may well be cytotoxic.1 However, in this issue of Circulation Research, McLean and colleagues2 demonstrate that endothelial dysfunction resulting from reperfusion injury is selective. One novel pathway, protease-activated receptor (PAR)–mediated coronary vaso-dilation, remains intact, making it a potential therapeutic target to maintain coronary blood flow.

PARs are cell surface membrane receptors activated by the serine proteases thrombin and trypsin. Thus, thrombin and trypsin, in addition to their well-characterized proteolytic activity, play an integral role in cell signaling.3 PARs are G protein–coupled transmembrane receptors activated by the thrombin-sensitive cleavage site, both found on the N-terminal extracellular domain. To date, 4 such receptors (PAR1, -2, -3, and -4) have been identified, each displaying a C-terminal intracellular tail and a long hydrophobic domains that criss-cross the cell membrane. They possess a C-terminal intracellular tail and a long N-terminal extracellular domain. To date, 4 such receptors (PAR1, -2, -3, and -4) have been identified, each displaying a similar mechanism of protease-dependent activation.

PAR1 is an important receptor for thrombin-mediated platelet activation, although PAR3 and PAR4 are also involved.4 PAR1 has a hirudin-like binding site for thrombin and a thrombin-sensitive cleavage site, both found on the N-terminal extracellular domain. Proteolytic cleavage of PAR1 reveals a novel amino terminus, which acts as a tethered ligand binding intramolecularly to effect transmembrane signaling. Short peptides corresponding to this exposed amino terminus can activate the receptor and have proven useful research tools. Receptor activation is irreversible; however, a novel mechanism of receptor desensitization involving phosphorylation, G protein–uncoupling, endocytosis, and lysosomal degradation prevents continuous cell signaling. This unusual pathway is critically dependent on the receptor’s cytoplasmic tail, as its substitution with the cytoplasmic tail of other G protein–coupled receptors prevents lysosomal degradation.5 Furthermore, recycling of unactivated receptors between an intracellular pool and the plasma membrane without degradation means that up to 50% of PAR2 is available for mobilization to the surface at any time, maintaining a fresh supply of signaling protein.

PARs display differential tissue expression. PAR1, the prototypic receptor cloned in 19916 is expressed on human platelets and vascular endothelial cells. PAR2, identified in 1994,7 is widely distributed, being found on vascular endothelial cells, smooth muscle cells, bronchial endothelium, cells of the gastrointestinal tract, sensory neurons, neutrophils, and eosinophils. Besides trypsin-induced activation, PAR2 may also be activated by synthetic oligopeptides mimicking the tethered ligand, mast cell-derived tryptase,8 tissue factor/factor VIIa complex, and factor X.9 The receptor is also cleaved by a recently identified cell membrane protease, membrane-type-serine protease (MT-SP)10 a potentially important ligand as the two proteins have similar tissue expression.

PAR2 is an integral component of the inflammatory process, and significantly, its expression by human endothelium is upregulated by cytokines.11 The diffuse distribution of PAR2 across cells from multiple systems belies its potential role in both local and systemic inflammation. PAR2 activation is involved in leukocyte recruitment, rolling, and adherence, possibly by a mechanism involving platelet-activating factor (PAF).12 PAR2 activation, and its response to inflammation, appears to occur without prostaglandin or NO involvement. Introduction of PAR2-activating peptide into a rat paw results in granulocyte infiltration and edema, neither of which requires prostaglandin or NO generation.13

In the cardiovascular system, endothelial expression of PAR2 is upregulated in a number of conditions, including atherosclerosis and vascular injury as outlined by McLean and colleagues.2 Given its activation by coagulation enzymes, PAR2 could be a component of the oft-noted link between inflammation and thrombosis in coronary artery disease. It may also play a role in vascular tone and blood pressure regulation as it is expressed on both vascular endothelium and smooth muscle cells.

Previous studies in several species have demonstrated significant heterogeneity in the vascular response to PAR2 activation. However, it appears that the disease state of the tissue, and specifically the local inflammatory environment, play a major role in influencing this response. Exposure of human coronary arteries to interleukin-1α (IL-1α) or tumor necrosis factor-α (TNFα) increases PAR2 and PAR4 expression, and significantly, IL-1α exposure promotes endothelium-
dependent relaxation induced by PAR2 and PAR4 synthetic peptides, but not by the PAR1 peptide.14

These findings are relevant during reperfusion of ischemic myocardium, where endothelial injury occurs as a result of both sudden free radical generation and the inflammatory response, characterized by adhesion and sequestration of neutrophils and lymphocytes. One mechanism of neutrophil migration and adhesion recently described involves de novo synthesis of interleukin-8 (IL-8) and intercellular adhesion molecule (ICAM) expression by ischemic endothelial cells.15

Also during reperfusion, NO-dependent endothelial pathways are adversely affected, notably, regulation of neutrophil adhesion and vasoconstriction. In another study,16 PAR2 expression in the rat heart was assessed following in vitro ischemia/reperfusion. There was no change in its expression in the ischemic heart; however, infusion of PAR2-activating peptide (SLIGRL) accelerated recovery of left ventricular function after reperfusion. Coronary flow was modestly increased in response to this peptide and the oxidant injury was attenuated.

McLean and colleagues2 evaluate the effect of PAR2 activation on post-ischemia coronary perfusion in the rat model clarifying the beneficial mechanism. Their article has 4 main findings. First, activation of PAR2 with trypsin or with activating peptide induces coronary vasodilation in an endothelial dependent manner, even in the normal coronary circulation. Interestingly, trypsin exhibited a biphasic dose-dependent curve, probably reflecting activation of PAR2 alone at low doses, but both PAR2 and PAR1 at higher concentrations. Alternatively, the response may reflect PAR transactivation, previously described with thrombin in human umbilical vein endothelial cells.17 Second, vasodilation in response to PAR2 activation was still evident after inhibition of cyclooxygenase and NO synthase, indicating involvement of another endothelial factor (most probably the elusive endothelial-derived hyperpolarizing factor based on experiments with pharmacological probes). This unique finding highlights the difference between small blood vessels (the major determinant of coronary resistance in the isolated rat heart preparation) and large blood vessels, where the response to PAR2 activation is at least in part dependent on endothelial-derived NO. This is also relevant to the study’s third finding, that PAR2 activation induced vasodilation after ischemia/reperfusion when NO-dependent pathways were lost. Finally, the authors propose an interesting mechanism for PAR2-induced vasodilation involving C-fibers innervating the coronary vasculature. We know that PAR2 is expressed on neurons and has been implicated in nociception.18 However, the authors indicate that PAR2-induced vasodilation is not due to direct interaction with nerve fibers but rather is mediated by a factor derived from endothelial cells that activates vanilloid (VR1) receptors on C-fibers. This may be a lipoxygenase-derived product of arachidonic acid, as the response can be partially reproduced by 12HpETE and blocked by lipoxygenase inhibitors. Admittedly, the evidence is indirect and the product was not identified; yet the work points to an intriguing PAR2-dependent mechanism in the microcirculation.

McLean and colleagues’ work2 demonstrates that post-ischemia coronary reperfusion involves an endothelial-dependent PAR2 mechanism. When extrapolating this result to the human model, there are several caveats to consider. First, although coronary reperfusion may now be achieved directly, it is most often induced by plasminogen activators, which trigger a cascade of enzyme activation, including enzymes of the coagulation pathway. Not only do these enzymes activate PAR2 and plasmin but they also degrade PARs nonspecifically. Other enzymes such as staphylocokine and streptokokase-plasmin complex, which may be administered therapeutically or generated by infection, cleave/activate PAR119 and could therefore have a similar effect on PAR2. Thus, the receptor may be inactivated and incapable of responding to ligands, natural or synthetic. One should also consider that, given the complex role of PAR2 in inflammation, its role in an acute event, such as an ischemic injury, may differ considerably from that in a chronic setting. Its potential pathological contribution to chronic inflammatory conditions of the lung, gastrointestinal tract, and nervous system is already being explored.

The identification of a human PAR2 variant where phenylalanine at position 240 is switched to a serine has functional significance. The residue lies close to the second extracellular loop, the putative binding site for the tethered ligand. The variant shows altered responses to ligands, displaying reduced sensitivity to trypsin and increased sensitivity to a selective PAR1 agonist.20 Exploring an association between this variant and patient outcome in thrombolysis trials would help to further relate the exciting findings of McLean and colleagues to human disease.

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References


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