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Review

Functional role of protease activated receptors in vascular biology

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ABSTRACT

Protease activated receptors (PARs) are a small family of G protein-coupled receptors (GPCR) mediating the cellular effects of some proteases of the coagulation system, such as thrombin, or other proteases, such as trypsin or metalloproteinase 1. As the prototype of PARs, PAR1 is a seven transmembrane GPCR that, upon cleavage by thrombin, unmasks a new amino-terminus able to bind intramolecularly to PAR1 itself thus inducing signaling. In the vascular system, thrombin and other proteases of the coagulation-fibrinolysis system, such as plasmin, factor VIIa and factor Xa, activated protein C, are considered physiologically relevant agonists, and PARs appear to largely account for the cellular effects of these enzymes. In the vasculature, PARs are expressed on platelets, endothelial cells (ECs) and vascular smooth muscle cells (VSMCs). In the vessel wall, under physiological conditions, PARs are mainly expressed in ECs and participate in the regulation of vascular tone, by inducing endothelium-dependent relaxation. PAR activation on ECs promotes conversion of these cells into a proinflammatory phenotype, causes increase of vascular permeability, and the exposure/secretion of proteins and cytokines mediating the local accumulation of platelets and leukocytes. These effects contribute to the vascular consequences of sepsis and of diseases such as acute lung injury and acute respiratory distress syndrome. In normal arteries PARs are to a much lesser amount expressed on VSMCs. However, in conditions associated with endothelial dysfunction, PARs mediate contraction, proliferation, migration, hypertrophy of VSMCs and their production of extracellular matrix, thereby contributing to the pathophysiology of atherosclerosis and hypertension. Inhibition of protease–PAR interaction might thus become a potential therapeutic target in various vascular diseases.

Contents

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1. Introduction

The serine protease thrombin plays a central role in the coagulation cascade and thrombosis. Thrombin is one of the most powerful physiological agonists in the cardiovascular system, and its actions are crucial for both atherosclerosis and its thrombotic consequences. Thrombin converts fibrinogen to fibrin, which is essential for providing the meshwork for clot formation.

In addition, thrombin also stimulates a wide range of cell types in blood and in the vasculature, including platelets, endothelial cells (ECs) and vascular smooth muscle cells (VSMCs). Therefore, thrombin acts both as an enzyme and as a receptor agonist. However, thrombin does not behave as a traditional agonist, as the catalytic activity of the enzyme is necessary to elicit the effects on cells. In fact, most of the cellular effects of thrombin are initiated via activation of a family of G-protein-coupled receptors (GPCR) termed protease-activated receptors (PARs). These receptors are characterized by a unique mechanism of activation, whereby the receptor undergoes proteolytic cleavage unmasking a new tethered aminoterminus (N-terminus) which autoactivates the receptor itself [1].

In this issue of *Vascular Pharmacology*, Gonzales et al. show that thrombin-induced increased endothelial permeability can be attenuated by heparin and a desulfated heparin molecule with low anticoagulant activity (ODSH) in a PAR-dependent manner [2]. The increased endothelial permeability by thrombin is relevant in severe human diseases such as acute lung injury (ALI) and acute respiratory distress syndrome (ARDS). Therefore, understanding the role of thrombin and PARs in the vasculature under physiological conditions and in diseases can be important for the clinical development of new therapeutic strategies.

The purpose of this review is therefore to briefly describe how PARs signal to vascular cells upon protease activation and their role in regulating vascular function under physiological and pathological conditions. Much knowledge has been acquired so far on thrombin and its major receptor PAR1 in normal and pathologic vessels. However, the other PARs and the proteases signaling through these receptors, other than thrombin, will also be described. The potential role of PARs as pharmacological targets in human vascular diseases will be discussed as well.

2. Protease activated receptors

2.1. Mechanism of PAR1 activation

In addition to cleaving fibrinogen and other soluble protein substrates, the protease thrombin triggers several responses in cells such as platelets, endothelial cells and other cells, thus behaving like a traditional hormone. How thrombin elicits cellular responses and regulates cellular behavior, by exerting its enzymatic activity, was a vexing question until the discovery of PARs. In 1991 Coughlin and coworkers used an expression cloning screen in *Xenopus* oocytes to identify the first thrombin receptor, a seven-member transmembrane GPCR characterized by a novel signaling mechanism, unique among GPCRs [3]. Initially, mRNA from cells highly responsive to thrombin was injected into *Xenopus* oocytes and the response to thrombin assayed. Fractionation of the mRNA encoding the receptor led to the construction of a size-specific cDNA library. By injecting in vitro transcribed cRNA into *Xenopus* oocytes and functionally assaying the responses to thrombin, eventually a single cDNA species was isolated which, when assayed in oocytes, displayed a strong and specific response to thrombin rather than trypsin [3]. This cloned thrombin receptor was henceforth identified as PAR1.

PAR1, the prototype of a small family of PARs, is activated when thrombin cleaves its N-terminal exodomain at a specific cleavage site, between residues Arg 41 and Ser 42. Cleavage by thrombin unmasks a new N-terminus, which acts as a tethered ligand, binding intramolecularly to the body of the receptor to effect transmembrane signaling (Fig. 1). A synthetic peptide mimicking the first six amino acids of the new N-terminus generated by the thrombin cleavage, SFLLRN – also known as thrombin receptor activating peptide (TRAP) – can activate PAR1 independent of protease activity and receptor cleavage. The novelty of the mechanism of PAR1 activation, in comparison with that of all other known receptors, is that in this case the receptor itself carries its own ligand, which is unmasked only upon enzymatic cleavage by thrombin at a specific site.

The 51–63 PAR1 region is rich in anionic residues and has a sequence resembling the C-terminal tail of the leech anticoagulant hirudin. This sequence is the primary binding site between PAR1 and the anion binding exosite I of thrombin (Fig. 2), and substantially increases the cleavage of PAR1 at low concentrations of thrombin. In fact, the k_{cat}/K_m value for the PAR1 hydrolysis is increased 100-fold in the presence of the hirudin-like domain [4].

Once ligated by the new N-terminus, PAR1 can activate heterotrimeric G proteins of the G12/13, Gq and Gi/z families to involve a substantial network of signaling pathways [5]. The α-subunits of G12 and 13 mediate cytoskeletal responses that likely induce the shape change in platelets [6] and increased permeability and migration in ECs [7] through small G-proteins such as Rho. Gαq activates phospholipase Cβ, triggering phosphoinositide hydrolysis, calcium mobilization and activation of protein kinase C [8]. This pathway mediates responses ranging from granule secretion to integrin activation and aggregation in platelets, and transcriptional responses in endothelial and mesenchymal cells through Ca-regulated kinases and phosphatases, as well as MAP kinase cassettes. Gαi signaling in platelets mediates adenylate cyclase inhibition, which can also be mediated by PAR1-mediated ADP secretion and $P2Y_{12}$ activation. Gβγ subunits can activate phosphoinositide-3 kinase (PI3-kinase) and other lipid modifying enzymes, protein kinases and channels [9]. PI3-kinase modifies the inner leaflet of the plasma membrane to provide the attachment and recruitment of several signaling proteins [10]. Therefore, PAR1 coupling to Gq, G13 and Gz may account for most of pleiotropic effects of thrombin on cells.

2.2. A family of PARs

After the discovery of the first thrombin receptor, now named PAR1, it was surprising to find out that platelets from PAR1 deficient mice responded strongly to thrombin [11] and the search for a second receptor for thrombin, at least in mice, began. By contrast, human PAR1 was able to elicit most thrombin responses in platelets. A second proteolytically activated seven transmembrane GPCR, whose DNA was isolated from a mouse genomic library and expressed in *Xenopus* oocytes, was identified and named PAR2 [12]. PAR2 could not be activated by thrombin, but it was activated by low dose of trypsin. A polymerase chain reaction (PCR)-based strategy yielded a new human complementary DNA encoding a putative GPCR with 27% amino-acid sequence similarity to the PAR1 and 28% similarity to PAR2, identifying what is now called PAR3 [13]. This new GPCR was cleavable by thrombin, triggered signaling in cells transfected with PAR3 cDNA, and was expressed in human bone marrow and in mouse megakaryocytes. However, thrombin responses in