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Physiology, pharmacology, and therapeutic potential of protease-activated receptors in vascular disease

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ABSTRACT

It has been 20 years since the discovery of the prototypical protease-activated receptor (PAR). In the time since this landmark work, significant advances have been made in our understanding of this family of four G protein-coupled receptors. Initially discovered and characterized in an attempt to determine the mechanism by which thrombin activates platelets, PARs have since been found to be widely expressed throughout the body, respond to multiple proteases, and to be involved in a vast array of physiological processes. Yet despite their wide-ranging expression, the function of PARs has been most extensively studied in the vascular system. In particular, the importance of PAR1 for platelet activation during arterial thrombosis has been thoroughly investigated and has led to the development of a host of PAR1 antagonists—two of which are currently in Phase 3 trials as antiplatelet agents. Given the impending clinical use of the first PAR antagonists, it is timely to review the physiological roles of PARs in cells of the blood and blood vessels, the development and experimental use of PAR antagonists in these systems, and the potential clinical application of these agents as therapeutics for vascular diseases.

Contents

1. Introduction

Serine proteases had long been observed to elicit intracellular signalling events and induce cellular responses in a variety of cells before the mechanism by which they achieved these effects was elucidated. In 1991 the landmark study from Shaun Coughlin's group used an expression cloning screen to identify the first human thrombin receptor, now known as protease-activated receptor 1 (PAR1) (Vu et al., 1991). This receptor class numbers four in mammals, assigned PARs 1–4 (Coughlin, 2000). PARs are G protein-coupled receptors found on the surface of cells from a wide variety of tissues. In the vascular system, PARs are expressed on platelets, most (if not all) leukocytes, vascular endothelial and smooth muscle cells, cardiomyocytes, cardiac fibroblasts, and nerves innervating the heart and blood vessels (for detailed PAR expression see Fig. 1 and also (Vu et al., 1991; A. J. Connolly et al., 1996; Bono et al., 1997; Ishihara et al., 1997; D'Andrea et al., 1998; Dery et al., 1998; Molino et al., 1998; Coughlin, 2000; Barnes et al., 2004).

The structure, activation mechanism, and signalling of PARs has been reviewed extensively elsewhere (Dery et al., 1998; Coughlin, 2000, 2005; Hamilton, 2009; Soh et al., 2010). Briefly, genes encoding human PARs 1–3 are located on chromosome 5(q13), and the gene for human PAR4 is on chromosome 19(p12). In the mouse, PARs 1–3 are on chromosome 13(p2), with PAR4 on chromosome 8(B3). Despite the distinct location of PAR4, all four genes display a high degree of

Abbreviations: PARs, Protease-activated receptors; PAR-AP, Protease-activated receptor activating peptides; TBD, Thrombin-binding domain; GPCR, G protein-coupled receptor; ADP, Adenosine di-phosphate; TxA_2, Thromboxane A2; VWF, Von Willebrand Factor.

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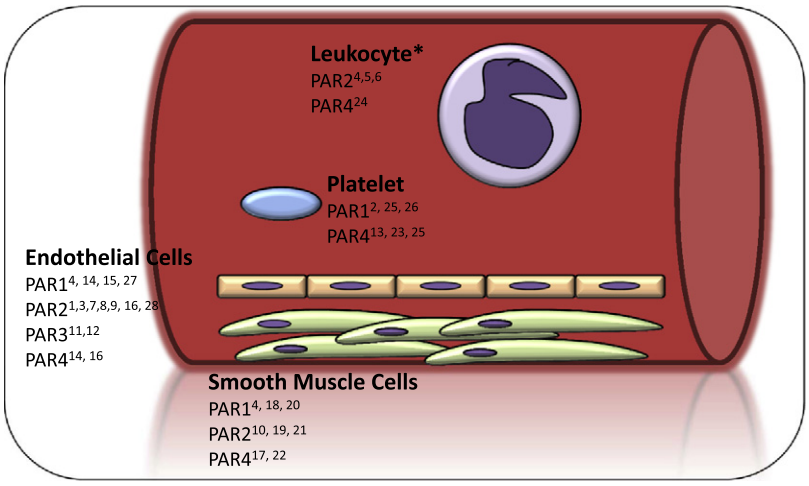

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3. D'Andrea *et al.*, 1998
4. Colotta *et al.*, 1994
5. Grandaliano *et al.*, 1994
6. Howells *et al.*, 1993
7. Bohm *et al.*, 1996
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9. Mirza *et al.*, 1996
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12. Molino *et al.*, 1998
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14. Kataoka *et al.*, 2003
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17. Vidwan *et al.*, 2010
18. Bachhuber *et al*, 1997
19. Kumuro *et al., 1997*
20. Dabbagh *et al.*, 1998
21. Damiano *et al.*, 1999
22. Hollenberg *et al.*, 1999
23. Sambrano *et al.*, 2000
24. Vergnolle *et al.*, 2002
25. Kahn *et al.*, 1999
26. Andersen *et al.*, 1999
27. Hamilton & Cocks, 2000
28. Hamilton *et al.*, 1999

Fig. 1. PAR expression in the human vascular system. PARs are expressed on various cell types in the blood and blood vessels and facilitate a wide range of cellular responses. Activation of PAR1 and PAR4 on platelets causes platelet activation, secretion, and aggregation. Activation of endothelial cell PAR1 (and in inflammatory conditions, PAR2 and PAR4) causes vasodilation and consequent hypotension, as well as inducing endothelial cell retraction and increasing vascular permeability. Activation of vascular smooth muscle cell PAR1 leads to proliferation and hypertrophy, and is generally pro-inflammatory. *PARs are expressed on virtually all leukocytes.

structural similarity, an indication that the overall structure and function of these receptors is conserved (Bohm et al., 1996; Kahn et al., 1998a; Xu et al., 1998). In both mice and humans, all four PARs have two exons: the first encoding a signal peptide with the second exon encoding the entire functional receptor protein (Kahn et al., 1998a). The prototypical PAR, human PAR1, is a 425 amino acid GPCR with a particularly long N-terminal extracellular domain containing several features important for the normal functioning of the receptor (Vu et al., 1991; Grand et al., 1996). Firstly, a thrombin cleavage site is present between amino acid residues 41 and 42 [LDPR^{41} / ^{42}SFLLRN] (Vu et al., 1991). Secondly, a hirudin-like thrombin-binding domain lies between residues 53 and 64 [DKYEPF], and is the primary binding site between PAR1 and the anion-binding exosite I of thrombin (Liu et al., 1991). Alterations to the thrombin-binding site of PAR1 substantially reduce the efficacy of thrombin cleavage of the receptor (Liu et al., 1991). Cleavage of PAR1 reveals a neo-N-terminus, starting now with the amino acid sequence ^{42}SFLLRN^{47}. This region of the receptor is known as the "tethered ligand", as it activates the receptor by binding to its own second extracellular loop (Vu et al., 1991). Critical interactions required for receptor activation include R^{46} of the tethered ligand with L^{260} of the second extracellular loop of the receptor. This "self-activation" then leads to conformational change of the receptor and the subsequent interaction with G proteins that these conformational changes allow (Nanevicz et al., 1995), as occurs with other GPCRs (Kristiansen, 2004). Synthetic peptides that correspond to the sequence of the tethered ligand are capable of activating the receptor independently of N-terminal proteolysis, confirming the self-activation model and providing a useful experimental tool for the specific activation of PARs (Faruqi et al., 2000).

Each of the four PARs is activated via this mechanism, with some minor modifications in each case. PAR2 is the most functionally distinct receptor in the PAR family as it is the only PAR not cleaved by thrombin. PAR2 is most effectively cleaved by trypsin (Nystedt et al., 1994), tryptase (Molino et al., 1997b), coagulation factors VIIa and Xa (Camerer et al., 2000), and the membrane-bound serine proteases MTSP1 (Takeuchi et al., 2000) and TMPRSS2 (Wilson et al., 2005). Human PAR3 is activated in a very similar fashion to human PAR1, inasmuch as it is a thrombin cleaved receptor with a hirudin-like thrombin-binding domain downstream of thrombin cleavage site (Ishihara et al., 1997). Remarkably, mouse PAR3 does not signal upon thrombin cleavage, but functions instead via a unique co-factoring mechanism to support the activation of PAR4. Like human PAR3, mouse PAR3 binds thrombin via an N-terminal thrombin-binding domain and is cleaved by the protease at its consensus cleavage site. However, in contrast to human PAR3, mouse PAR3 is not activated in response to such cleavage. Indeed, while the expression of human PAR3 cDNA in either COS cells or *Xenopus oocytes* confers thrombin responsiveness in these cells, similar expression of mouse PAR3 cDNA fails to permit thrombin signalling. Rather, coexpression of mouse PAR3 and PAR4 enhances thrombin sensitivity in such expression systems when compared with the expression on mouse PAR4 alone (Nakanishi-Matsui et al., 2000). Strikingly, when the N-terminal exodomain of mouse PAR3 was inserted into the plasma membrane of these cells it was sufficient to reproduce this 'PAR4 sensitising' response, and mutation of the thrombin-binding domain in this sequence abolished the effect (Nakanishi-Matsui et al., 2000). On the basis of this work, and in contrast to the human receptor, mouse PAR3 appears unique among GPCRs in that it is incapable of mediating transmembrane signalling but functions as a co-factor for the cleavage and activation of mouse PAR4. In contrast to PAR1 and PAR3, PAR4 is a thrombin-sensitive receptor which lacks a thrombin-binding domain, such that PAR4 cleavage by thrombin is significantly less efficient than observed for the other receptors (Kahn et al., 1998b). Activation of PARs results in a multitude of cellular signalling events. Of the four major Gα protein subclasses, both PAR1 and PAR2 have been shown to signal through G_q, G_i, and $G_{12/13}$, PAR3 through G_q, and PAR4 through G_q and $G_{12/13}$ (Soh et al., 2010)—although the coupling of PAR1 and PAR4 to G_i remains controversial (Kim et al., 2002; Lova et al., 2004; Kim et al., 2006).

2. Physiology of PARs in the vasculature

2.1. Platelet PARs

Platelets are the main cellular component of arterial thrombi. The platelet surface expresses a number of adhesion receptors essential for the initial localization of platelets to sites of vascular damage, most notably receptors for the blood and vessel wall proteins vWF (glycoprotein (GP) complex Ib-V-IX), collagen (GPVI and the integrin $\alpha_2\beta_1$), and fibrinogen (integrin $\alpha_{IIb}\beta_3$). Localized platelets are then activated by a number of mediators released or formed locally, most