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Protease-activated receptor-2 (PAR_2) in cardiovascular system

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Abstract

Vascular system is constituted by a complex and articulate network, e.g. arteries, arterioles, venules and veins, that requires a high degree of coordination between different elemental cell types. Proteinase-activated receptors (PARs) constitute a recent described family of 7-transmembrane G protein-coupled receptors that are activated by proteolysis. In recent years several evidence have been accumulated for an involvement of this receptor in the response to endothelial injury in vitro and in vivo experimental settings suggesting a role for PAR_2 in the pathophysiology of cardiovascular system.

This review will deal with the role of PAR_2 receptor in the cardiovascular system analyzing both in vivo and in vitro published data. In particular this review will deal with the role of this receptor in vascular reactivity, ischemia/reperfusion injury, coronary atherosclerotic lesions and angiogenesis.
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Keywords: PAR_2; Vascular reactivity; Endothelium; G-protein

1. Introduction

Vascular system is constituted by a complex and articulate network that requires a high degree of coordination between different elemental cell types. Undoubtedly, the endothelial cells (EC) play a pivotal role in the vascular homeostasis being located at the interface between blood and the underlying smooth muscle cells. EC regulate complex functions compliant to specific necessities in time and location. Protease-activated receptor-2 (PAR_2) has been shown to be involved in vessel function. In particular it appears to play a role in pathological conditions. Thus, PAR_2 represents one of the most intriguing receptor since its physiological and physiopathological activity in the vasculature is not as yet well defined.

Proteinase-activated receptors (PARs) constitute a recent described family of 7-transmembrane G protein-coupled receptors that are activated by proteolysis (MacFarlane et al., 2001; Hollenberg and Compton, 2002). All PARs share a unique mechanism of activation where the serine proteases cleave at specific sites within the extracellular N-terminus to unmask a tethered ligand domain that interacts with receptor in the extracellular loop II initiating signalling. This process is irreversible since once cleaved PARs they cannot longer be used by the cell and are degraded terminating signalling (Cottrell et al., 2003). Thus far, only four receptors belonging to this family have been described: PAR_1, PAR_2, PAR_3 and PAR_4 (Hollenberg, 1999). The thrombin receptor PAR_1 was the first PAR to be discovered and cloned (Vu et al., 1991). In 1994, Nystedt identified PAR_2 cloning a mouse genomic DNA encoding a proteolitically activated receptor, similar to PAR_1 with a different sequence (Nystedt et al., 1994); successively PAR_3 and PAR_4 have been identified (Ishihara et al., 1997; Kahn et al., 1998). While the ligand of PAR_1, PAR_3 and PAR_4 has been identified in thrombin, the physiological agonist of PAR_2 remains elusive, however trypsin seems to be the major candidate as PAR_2 specific ligand since it cleaves the receptor within the sequence N_{34}/P_{48} of its extracellular $-NH_2$ terminus, unmasking the tethered sequence SLIGRLETQ... responsible of the receptor autoactivation. Besides trypsin, it has been shown that PAR_2 can be activated by tryptase (Molino et al., 1997a,b) and factor Xa (Fox et al., 1997), but it is not activated by thrombin even at concentrations as high as 100 nM (Cicala, 2002). In absence of proteolysis all PARs, with the

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exception of PAR_3, can be activated by specific synthetic peptides (PARs-AP) which, mimicking the specific tethered ligand sequence can activate the receptor without causing proteolysis (Dery et al., 1998).

2. PAR_2 signalling

Although it has been demonstrated a wide tissue distribution of PAR_2, in particular in mammalian tissue (Pearson et al., 2001), there are not much studies examining PAR_2-mediated intracellular signalling compared to PAR_1. An explanation of this could be imputable to the relative low degree of expression of PAR_2 in basal condition when compared to PAR_1. The involvement of PAR_2 activation is well documented much more in physio-pathological than in physiological environment. Indeed, a role for PAR_2 has been proposed in proliferative (Mirza et al., 1996; Akers et al., 2000; Frungieri et al., 2002; Gaca et al., 2002) and inflammatory processes (Wakita et al., 1997; Cicala et al., 1999; Vergnolle et al., 1999, 2001; Ferrell et al., 2003). A linkage between PAR_2, SAP-kinase, JNK, and P38 MAP kinase has been found in cardiac myocytes and in transfected skin epithelial cells (Belham et al., 1996; Sabri et al., 2000), indicating that PAR_2 activation can be involved in proinflammatory responses (MacFarlane et al., 2001). In addition, it has been also demonstrated that trypsin and PAR_2AP stimulate nuclear factor kB pathway in keratinocyte cell line and in coronary smooth muscle cells (Bretschneider et al., 1999; Kanke et al., 2001) further supporting a direct involvement of PAR_2 activation in proinflammatory cellular signalling (Table 1). It is well established that PAR_2 activation by trypsin or PAR_2AP induces an increase of intracellular calcium together with the production of IP_3 and DAG (Nystedt et al., 1995; Santulli et al., 1995). This pathway acts through activation of PLC isoforms by using Gq/G_{11} coupled receptor modulating several intracellular targets (Macfarlane and Plevin, 2003). The increase of intracellular calcium upon PAR_2 stimulation is fast in onset and returns to baseline levels within 2 min (Kawabata et al., 1999; Laniyonu and Hollenberg, 1995). This transient calcium response is typical of PARs receptor (at least PAR_1 and PAR_2), since activation of other G-protein-coupled receptors, such as α-adrenoreceptor, give more prolonged responses (Hollenberg and Compton, 2003). Activation of PAR_2 leads also to G_0/G_i dependent transduction mechanism as demonstrated by the PTX-sensitive calcium modulation in Xenopus oocytes stimulated with trypsin (Schultheiss et al., 1997) and in HEK-293 transfected with PAR_2 stimulated with PAR_2-AP (Yu and Hinkle, 1997). The response of HEK-293 transfected with PAR_2 receptor leads to tyrosine phosphorylation of SHP-2, a tyrosine phosphatase previously observed to play a role in PAR_1 mitogenic signalling (MacFarlane et al., 2001).

Another peculiar characteristic of PAR receptors consists in a very rapid desensitization coupled to a receptor internalization processes (Bohm et al., 1996; De Fea et al., 2000). For what concerns PAR_2 it is known that the receptor is internalised through clathrin-coated pits to the endosomes followed by redistribution to lysosomes (Dery et al., 1999). This phenomenon seems to be coupled to specific signalling involving MAPkinase/ERK1, 2 (De Fea et al., 2000). Signal desensitization and receptor internalization is also controlled by phosphorylation of the C-terminal domain of the receptor by kinase C (Ishii et al., 1994; Bohm et al., 1996) or by G-protein-coupled-receptor kinases (Krupnick and Benovic, 1998). The phosphorylated receptor can thus interact with β-arrestin and dynamin more efficiently and in turn cause desensitization/internalization (Ishii et al., 1994; Bohm et al., 1996).

The presence of a putative N-linked glycosilation site in the extracellular domain is a common characteristic of all PAR members so far discovered (Compton, 2003). Glycosilation shifts $hPAR_2$ molecular weight from 45 kDa up to 60 kDa. At least one of these N-linked sites appears to be required for efficient cell surface expression of PAR_2 (Compton et al., 2002). Expression of $hPAR_2$ is only partially inhibited in HEK293 cells by using tunicamicin, an inhibitor of cellular glycosilation machinery, suggesting that receptor glycosylation is important but not essential for PAR_2 expression in this context (Compton et al., 2001). It has been speculated that N-linked glycosylation, and in particular the sialylation (Kitagawa and Paulson, 1994), plays a role in tryptase-induced PAR_2 activation (Compton et al., 2001). Indeed, differentially glycosylated PAR_2 may be responsible for the failure of tryptase responses observed in several previous studies (Molino et al., 1997a,b; Schechter et al., 1998; Huang et al., 2001).

Table 1
Pathways involved in PAR_2 signal transduction

Cell type	Signaling	G protein involved	References
Cardiomyocytes transfected skin epithelial cells	SAPk JNK P38MAPk	G_q/G_{11}	Belham et al., 1996; Sabri et al., 2000
Human keratinocytes	▲ IP3 ▲ DAG ▲ $[Ca^{2+}]_i$	G_q/G_{11}	Nystedt et al., 1995; Santulli et al., 1995
Xenopus oocytes	▲ $[Ca^{2+}]_i$	Go/G_i	Schultheiss et al., 1997
Transfected HEK-293	▲ c-*fos*	Go/G_i	Yu and Hinkle, 1997; MacFarlane et al., 2001
Keratinocytes cell line	NF-kB		Bretschneider et al., 1999; Kanke et al., 2001
Coronary smooth muscle cells			

3. Involvement of PAR_2 in vascular homeostasis

PAR_2 is widely expressed in several tissues such as kidney, stomach, pancreas, intestine, bladder, exocrine glands, airway, bones, epidermis and brain; in particular it is present in endothelial and epithelial cells, myocytes, fibroblasts, neurons, glial cells and immune cells (for review see MacFarlane et al., 2001). Northern blot analysis performed on murine tissues demonstrated receptor transcripts in highly vascularized organs such as kidney, small intestine, and stomach (Nystedt et al., 1994). In human species immunohistochemical studies provided a tissue-specific cellular localization of

PAR_2 in normal tissues by using a polyclonal antibody raised against a peptide corresponding to the amino terminal sequence SLIGKVDGTSHVTGKGV of PAR_2 (D'Andrea et al., 1998). Strong immunolabeling was observed in smooth muscle of vascular and nonvascular origin from a variety of tissues, in endothelial and epithelial cells independent of tissue type, in epidermis, throughout the gastrointestinal tract and, in central nervous system, in astrocytes and neurons (D'Andrea et al., 1998).

3.1. In vitro pharmacology

Al-Ani and colleagues first described a functional role for PAR_2 on endothelial cells in 1995. They showed that trypsin and the PAR_2 peptide agonist SLIGRL-NH2 induced an endothelium dependent vasorelaxation in rat aortic rings. The vasorelaxant effect was reduced by L-NAME, an inhibitor of nitric oxide (NO) synthases, supporting an involvement of the L-arginine/NO pathway (Al Ani et al., 1995). Successively, endothelium NO dependent response following PAR_2 activation has been demonstrated in several other blood vessels such rabbit aorta (Roy et al., 1998), porcine coronary (Hwa et al., 1996; Hamilton et al., 1998) and basilar arteries (Sobey and Cocks, 1998; Sobey et al., 1999). Since inhibitors of the L-Arginine/NO pathway did not abrogate the vasodilatory response induced by PAR_2 it has been investigated the possible involvement of other mediators. On this basis it has been proposed endothelin as second messenger. Indeed, rapid release of nitric oxide induced by stimulation of aortic rings with PAR2 agonist, SLIGRL-NH_2, was reduced by pre-treatment with BQ-788, an ET_B endothelin receptor-specific antagonist. Consistent with a role for endothelin-1 receptor activation in PAR_2AP-induced NO release, endothelin-1 levels were increased significantly after 5 min of treatment of aortic rings with PAR_2AP. These results strongly support an involvement of ET_{1B} receptor in PAR_2 response (Magazine et al., 1996). Similarly, it has been shown that NO production did not entirely account for vasorelaxant action of PAR_2AP in resistance vessels (Hamilton and Cocks, 2000) such as rat femoral artery, vein (Emilsson et al., 1997; Roy et al., 1998) and isolated perfused normal rat kidney (Trottier et al., 2002). In all these cases PAR_2-induced transient vasorelaxation persisted even after removal of the NO component of PAR_2-induced vasodilatation, by blocking nitric oxide synthase. This evidence has lead to hypothesize an involvement of endothelium hyperpolarizing factors (EDHFs) in PAR_2-induced vasodilatation. This hypothesis is supported by the finding that afferent arterioles preconstricted with elevated concentration of KCl do not relax to PAR_2AP (Trottier et al., 2002). Consistent with this hypothesis, on mouse second-order mesenteric arterioles, PAR_2-induced vasorelaxation is endothelium-dependent and inhibited by either 30 mM KCl-precontraction, or pre-treatment with apamin, charybdotoxin, or their combination. These results further support a role for endothelium-dependent hyperpolarization factor in PAR_2-induced relaxation that could involve activation of an apamin/charybdotoxin-sensitive potassium channels (McGuire et al., 2002). More recently, it has been shown that in isolated rat gastric artery receptor activating peptides for both PAR_1 and PAR_2 produce endothelium-dependent vasorelaxation. The multiple mechanisms underlying the PAR_1 and PAR_2-mediated vasodilatation confirm the involvement of NO, EDHF and prostanoids (Kawabata et al., 2004). All these evidences indicate that redundant signalling pathways contribute to the vasodilatory response following PAR_2 activation as it has been underlined by a recent study on afferent arterioles (Wang et al., 2005). On the other hand, this redundancy in PAR_2 signalling cascade could play a role in pathological settings such as inflammation or ischemia in which PAR_2 is thought to be activated. Conversely, while originally it was shown that removal of endothelium caused the loss of the vasodilatory effect of PAR_2 subsequent studies have shown that depending on the vascular district an effect can be evidenced. Thus, while PAR_2AP fails to cause contraction in aortic rings without endothelium (Magazine et al., 1996; Saiffedine et al., 1996; Emilsson et al., 1997) it contracts in endothelium denuded rabbit aorta (Komuro et al., 1997). In addition a vasoconstrictory effect PAR_2 mediated (but not PAR_1APs) has been shown in vitro on rat pulmonary artery (Roy et al., 1998) and human umbilical vein (Saiffedine et al., 1998). These findings have lead to propose that contraction could be mediated via a receptor distinct from PAR_2 through a release of a diffusible endothelial derived contracting factor (EDCF) that is different from previously recognized smooth muscle agonists such as prostanoid metabolites, endothelin, noradrenaline, angiotensin-II, acetylcholine (Saiffedine et al., 1998; MacFarlane et al., 2001).

All this in vitro work has been performed in order to define the physiological role of PAR_2 but the picture is rather confusing, what can be said is that PAR_2 activity is mainly vasodilatory and it looks like to be district dependent.

Studies performed in vitro simulating pathological conditions have suggested a possible protective role for PAR_2. A protective role for PAR_2 has also been proposed in myocardial ischemia/reperfusion (I/R) injury (McLean et al., 2002). In particular it has been shown a protective effect, for PAR_2 in the coronary vasculature. Indeed, in isolated and perfused rat heart SLIGRL-NH_2 peptide produced an endothelium-dependent coronary vasodilatation. Following after I/R injury, PAR_2AP-induced vasodilatation was selectively preserved as opposite to acetylcholine response. PAR_2 response was not mediated by NO or prostanoids, but involved the release of an EDHF, possibly a lipoxygenase-derived eicosanoid, and the activation of vanilloid receptors on sensory C-fibers (McLean et al., 2002).

3.2. In vivo pharmacology

Intravenous injection of SLIGRL-NH_2 or SLIGKV-NH_2 causes hypotension in anesthetized rats (Emilsson et al., 1997; Cicala et al., 1999) and mice (Cheung et al., 1998). The effect is dose-dependent, and has been shown do not involve central vasoregulatory systems, heart rate, or the kidneys. Systemic administration of L-NAME attenuates the hypotensive response induced by PAR_2APs further supporting PAR_2AP-induced NO release hypothesis. The relative role played by PAR_2 and PAR_1 in physiological conditions has been addressed by developing mice deficient in PAR_2 or PAR_1. In this key paper, it has been evaluated mean arterial pressure and heart rate (HR) changes in response to PAR_1 or PAR_2 activation in anesthetized wild-type (WT), PAR_1-deficient ($PAR\text{-}1^{-/-}$), and PAR_2-deficient ($PAR_2^{-/-}$) mice. In WT mice, SLIGRL-NH_2, a PAR_2 selective activating peptide, caused hypotension without changing HR and this response was virtually absent in PAR_2 $^{-/-}$ mice. In addition, the hypotensive and bradycardic responses caused by PAR_1AP in WT mice resulted accentuated in $PAR_2^{(-/-)}$. These data confirmed the in vivo specificity of PAR_1 and PAR_2 respective activating peptides, and the distinct hemodynamic responses mediated by activation of PAR_1 or PAR_2. Moreover, the accentuated response to PAR_1 activation in PAR_2 deficient mice suggests that a compensatory response and a potential receptor cross-talk exists (Damiano et al., 1999a).

As opposite to PAR_1, the notion that PAR_2 is involved in pathological more than physiological conditions is supported by recent literature, even if it is not well established if this receptor has a protective or detrimental role. A role for PAR_2 in endotoxin shock has been suggested. Indeed, anesthetised rats 20 h from lypopolisaccharide treatment display a powerful increase of the hypotensive response to PAR_2AP and trypsin. Immunohistochemistry showed a preferential and localised increased expression of PAR_2 in aorta and jugular vein associated to endotoxin shock (Cicala et al., 1999). A role in blood pressure control in pathological conditions has been also shown in spontaneously hypertensive rats (SHR). In this strain basilar artery displays, in vitro, an enhanced vasodilatation to PAR_2AP as opposite to normotensive. In both strains, response to PAR_2AP was abolished by inhibition of NO synthesis indicating that NO-mediated vasodilatation to PAR_2 activation is selectively activated and augmented in SHR. These results suggest a protective role for PAR_2 in the cerebral circulation during chronic hypertension (Sobey et al., 1999). A protective role for PAR_2 has been shown also in experimental myocardial ischemia–reperfusion injury. Indeed, PAR_2AP infusion causes a recovery of myocardial function and decrease in oxidation at reflow coupled to a reduction of the ischemic risk zone and of creatine kinase release (Napoli et al., 2000). An enhanced expression of PAR_2 has been also shown in human coronary atherosclerotic lesions implying that PAR_2 dependent cellular trafficking could play a role in regulating signalling to the vascular injury (Napoli et al., 2004). A role for PAR_2 in proliferation has been also evaluated in rat carotid artery subjected to balloon-catheter injury. Following the injury there was a clear increase in damaged or necrotic smooth muscle cells when compared to normal vessels. In addition, proliferating adventitial myofibroblasts and the media and neointima of injured vessels labelled strongly for PAR_2 (Damiano et al., 1999b). These data further suggest a possible protective or compensatory role for PAR_2 in damaged vessels.

In line with this hypothesis it has been shown that in mouse model of hind limb ischemia PAR_2AP and trypsin administration increased the reparative angiogenic response with an accelerated hemodynamic recovery and enhanced limb salvage. These data also are suggestive of a potential angiogenic property of PAR_2 (Milia et al., 2002). Recently, the involvement of PAR_2 in angiogenesis has been also demonstrated by Belting and colleagues (2004). In particular, it has been shown that PAR_2 signalling is downstream to tissue factor (TF)-VIIa protease complex and it is involved in promoting tumour and angiogenesis in mice and human ocular tissue (Belting et al., 2004).

Overall these data suggest a possible role for PAR_2 as an *accessory receptor* in cardiovascular inflammation; indeed it is

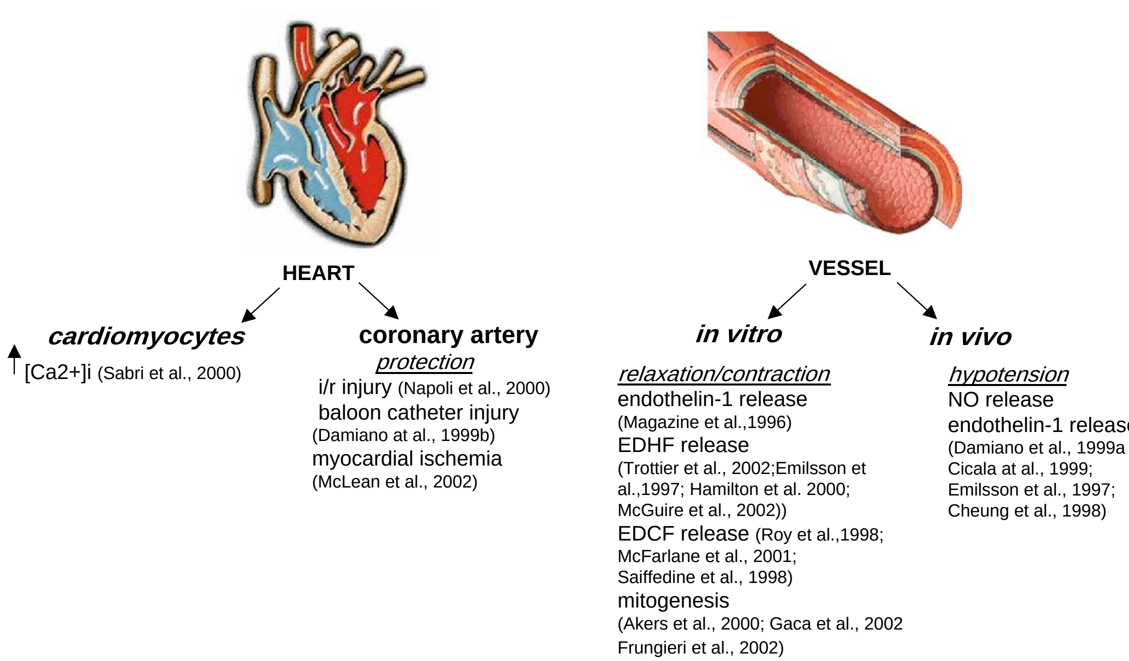

Fig. 1. In vitro and in vivo effects of PAR_2 activation.

silent in normal, basal physiological conditions while it is induced and *active* when endothelial damage occurs. However, it must be pointed out that in other context this receptor has been shown to be pro-inflammatory. Kawabata et al. (1998) firstly showed that PAR_2AP causes inflammation in rat paw (Kawabata et al., 1998). This original observation was confirmed in a more complete study by Vergnolle et al. (1999). In this study pre-treatment of rats with a cyclooxygenase inhibitor or a nitric oxide synthase inhibitor had no effect on the PAR_2AP-induced oedema demonstrating that the PAR_2-induced inflammatory response occurred through a mechanism largely independent of mast cell activation, and of the production of prostanoids and nitric oxide (Vergnolle et al., 1999). The same authors, by intravital microscopy, also showed that topical addition of PAR_2AP to rat mesenteric post capillary venules induced leukocytes rolling and adherence (Vergnolle et al., 1999). This effect was not affected by pre-treatment with a mast cell stabilizer or by prior degranulation of mast cells but was completely inhibited by pre-treatment with a platelet-activating factor receptor antagonist WEB 2086 (Vergnolle, 1999). These data suggest that PAR_2 activation could contribute to several early events in the inflammatory reaction, including leukocyte rolling, adherence, and recruitment, by a mechanism dependent on platelet-activating factor release. Recently it has been demonstrated an important role for PAR_2 in mediating chronic inflammation; in particular, using an adjuvant monoarthritis model, Ferrell et al. (2003) show that joint swelling is substantially inhibited in PAR_2 deficient mice (−/−) with a complete lack of joint damage. Overall these experimental data identifies in PAR_2 a potential novel target for the management of inflammation (Fig. 1).

4. Concluding remarks

At the present stage of the research no definitive conclusions can be drawn. However PAR_2 activation in the cardiovascular system appears to be a compensatory response rather than pathological event. Its tight linkage with L-arginine-NO pathway, demonstrated in several in vitro and in vivo setting, suggests a possible cross-talk between these systems. Indeed, in conditions where a vascular damage occurred PAR_2 expression has been shown to be enhanced. On the functional side PAR_2 stimulation, in these cases, causes a beneficial effect rather than a worsening of the disease. Conversely, activation of this receptor in other context appears to drive inflammation. These apparently contrasting results could be explained by the presence of another receptor or subtype of PAR_2, as it has been already proposed. Alternatively, it may be possible that PAR_2 is a receptor context-sensitive that depending upon the organ involved and/or the pathological conditions could be either detrimental or beneficial. This hypothesis is supported by the fact that different signal transduction have been proposed for this receptor depending on the experimental setting.

In conclusion “intriguing receptor” remains the most appropriate appellative for PAR_2 since its distribution, activation and signalling cascade are not unique but variable upon the environment.

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