Intracellular Signalling by the G-Protein Coupled Proteinase-Activated Receptor (PAR) Family

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ABSTRACT The proteinase-activated receptor (PAR) family are novel members of the G-protein coupled receptor superfamily that are activated by a mechanism involving specific proteolytic cleavage of their N-terminal. Activation of intracellular signalling pathways by the PAR family has received relatively little attention compared with the other aspects of their function. Along with activation of intracellular calcium, diacylglycerol, and inositol 1,4,5-trisphosphate, PARs have been reported to activate signalling pathways closely linked to both cell proliferation and inflammation. Here we attempt to describe these signalling pathways and the G-proteins involved in coupling the receptors to these intracellular events. Drug Dev. Res. 59:367–374, 2003. © 2003 Wiley-Liss, Inc.

Key words: PAR; G-protein coupled receptor signalling; MAP kinase; mitogenesis; inflammation

INTRODUCTION

In addition to the known role for thrombin in the blood-clotting cascade, various studies reported the enzyme had specific cellular effects [Chen and Buchanan, 1975; Bar-Shavit et al., 1983], the most prominent of which was the induction of cellular proliferation. The mechanism by which thrombin brought about these cellular effects was unknown; however, a sea change in the physiological role of thrombin occurred in 1991 with the cloning of a novel G-protein coupled receptor (GPCR), designated protease-activated receptor-1 or PAR₁. This new receptor was found to be activated by thrombin-mediated cleavage of the receptor protein and to have a wider range of expression than solely cell types involved in blood clotting [Vu et al., 1991]. Major advances have been made in our understanding of the pathophysiological roles played by this novel family of G-protein coupled receptors since the discovery of the PAR₁ receptor. However, insights into the intracellular signalling events that follow activation of these receptors have been less fully investigated, and are only now beginning to be unraveled. These signalling pathways provide the framework for the effects of receptor activation at a cellular level, and therefore understanding them is of considerable importance if the PAR family is to be viewed as important therapeutic targets. In this report, we discuss the intracellular signalling pathways identified as being activated by the PAR family, and attempt to link the activation of these pathways with the known function of the PARs in various tissue types.
PAR₁ AND G-PROTEIN INTERACTIONS

The prototypic member of that PAR family, PAR₁, has been the most widely studied of this family, not only in terms of receptor's general physiological role, but also with respect to intracellular signalling. Association of this seven transmembrane domain-receptor with heterotrimeric G-proteins has become well established, and PAR₁ has also been identified as influencing a wide range of intracellular signalling intermediates [Macfarlane et al., 2001].

G-proteins transduce signals to cells from receptors by coupling the receptors to a number of effector enzymes. Activation of these effector enzymes, such as adenyl cyclase, allows the receptors a wide sphere of influence within the cell by changing the activities in various intracellular signalling cascades. G-proteins fall into three groups of subunits, Gα, Gβ, and Gγ and their importance to intracellular signalling from seven transmembrane domain receptors has become an important area of study within pharmacology [Neves et al., 2002]. Included amongst the G-protein coupled receptor family are α- and β-adrenoceptors, muscarinic cholinoreceptors, and 5-HT receptors amongst others [Albert and Robillard, 2002]. This highlights the wide reliance and importance of this multifunctional form of signal transduction as these receptors often control vital cellular processes. G-protein α subunits are separated into four main subfamilies Gαs, Gαi, Gαq, and Gα12, with 27 genes encoding the various members of the α subunit families, 5 genes encoding β subunits, and a further 14 γ genes, and there is a wide scope for diverse heterotrimeric complexes to form [Albert and Robillard, 2002] and, therefore, many possibilities for interactions with signalling pathways.

PAR₁ was initially demonstrated to bring about two main signalling events. Firstly inhibition of adenyl cyclase and subsequent inhibition of cAMP production through interaction with inhibitory members of the Gα family [Hung et al., 1992; Kanthou et al., 1996] and, secondly, stimulation of phospholipase C (PLC) initiated inositol 1,4,5-trisphosphate production (IP₃) [Babich et al., 1990; Hung et al., 1992]. Through this pathway, the receptor can bring about both intracellular calcium release, via the action of IP₃ on inositol 1,4,5-trisphosphate receptors the endoplasmic reticulum (ER), and activation of members of the PKC superfamily through the generation of the endogenous PKC activator, diacylglycerol (DAG). Subsequently, more detailed studies have identified roles for Gαq/11, Gα12, and Go subunits in PAR₁ mediated signalling [Babich et al., 1990; Brass et al., 1991; Baffy et al., 1994; Ogino et al., 1996]. Taken together with reported effects via the G₁₂/₁₃ class of α subunit [Offermanns et al., 1994], these findings demonstrate that PAR₁ can activate a wide range of intracellular signalling pathways including the p42/44 MAP kinase via activation of c-Src, [NK and PI-3 kinase [Mitchell et al., 1990; Walker et al., 1998; Swift et al., 2000] (Fig. 1).

Traditionally it was believed that the βγ subunits of the G-protein family were predominant in the transduction of signals from receptors. However, a role for the βγ-subunits has been demonstrated and has been recently proposed in the signalling of PAR₁ to a number of signalling pathways including PI-3K (see below) and the inflammatory nuclear factor KB (NFkB) transcription factor signalling pathway [Rahman et al., 2002]. This transcription factor has previously been identified as being activated by PAR₁ [Nakajima et al., 1994; Bretschneider et al., 1997]. This more recent study adds some detail to the upstream signalling pathway utilized in the activation of this pathway. Another aspect of Gα subunit function is the activation of small molecular weight G-proteins such as Rac, Ras, and Rho [Matozaki et al., 2000]. The activation of members of this superfamily has been identified for PAR₁ with the identification of Rho activation via PTX-insensitive G₁₂/G₁₃, a mechanism implicated in regulation of cellular invasion in cancer [Nguyen et al., 2002].

PAR₁ COUPLING TO P42/44 MAP KINASE ACTIVATION

Activation of the MAP kinase family of proteins by PAR₁ has received much attention [Van Corven et al., 1993; Chen et al., 1996; Apostolidis and Weiss, 1997], with strong evidence for the coupling of PAR₁ to the Raf/MEK system of p42/44 activation via the Gα family. The activation of these important cellular kinases is well established for tyrosine kinase-linked receptors [Malarkey et al., 1995] and signalling paradigms for the linkage of G-protein coupled receptors to the MAP kinases have also been established. However, PAR₁ has been shown to use both activation of this pathway via Src and also by transactivation of the pathway via growth factor receptor kinases (see below) [Sabri et al., 2002]. Additionally, a role for the PI-3 kinase pathway has been identified in the activation of p42/44 MAP kinase [Malarkey et al., 1995; Touhara et al., 1995]. In addition to utilising the tyrosine kinase receptor associated protein isoform of PI-3 kinase p85, thrombin also activates the p110 kDa isoform that is directly activated by G-protein βγ subunits. Furthermore, the activation of PI-3 kinase signalling by thrombin has been implicated in downstream activation of important mitogenic and cell survival proteins such as p70S6K and protein kinase B [Belham et al., 1997; Krymskaya et al., 1999; Walker et al., 1998]. The potential
importance of PAR1 as a regulator of cellular proliferation is therefore further highlighted by the activation of these mitogenic proteins.

Transactivation has become an important concept in the activation of signalling pathways by G-protein coupled receptors [Daub et al., 1997; Maudsley et al., 2000]. This mechanism enables GPCRs to activate a cellular kinase that phosphorylates and activates a growth factor receptor, therefore allowing the GPCR to utilise the growth factor receptor signalling machinery. Research to date suggests that PAR1 uses this strategy not only to activate p42/44 MAP kinase [Weiss and Maduri, 1993; Delafofontaine et al., 1996], but also in the activation of the stress-activated protein kinase p38 MAP kinase [Kanda et al., 2001]. It should be noted, however, that the use of transactivation to activate p42/44 MAP kinase might be a cell-type specific event [Sabri et al., 2003; Wang et al., 2002]. In general, it is possible that transactivation pathways activated via G-protein coupled receptors represent late phase activation of the signalling pathways, allowing long-term signals to be maintained.

PAR1 is a well-established activator of multiple signalling pathways within cells, with many of these pathways directly involved in cell growth and differentiation. However, there is increasing evidence for PAR1 activation of the inflammatory transcription factor NFkB [Nakajima et al., 1994; Bretschneider et al., 1997; Rahman et al., 2002]. The actions of both thrombin and PAR1 agonist peptide have been linked to cell proliferation in vascular smooth muscle [Nakajima et al., 1994; Bretschneider et al., 1997], and PAR1 also increases the expression of adhesion molecules in vascular endothelial cells via a pathway involving NFkB [Minami et al., 2002]. The exact delineation of the signal from PAR1 that brings about activation of the NFkB dimer is, as yet, unidentified. However, recent work has pointed to the involvement of both PI-3 kinase and PKCδ in this event [Minami et al., 2002], although the role of the inhibitory kappaB kinase (IKK) isoforms, found upstream of NFkB, was not assessed. Furthermore, this study reported the activation of PKCζ by thrombin, leading to the activation of downstream GATA signalling pathways.

Some discrepancies have been observed between the signalling pathways activated by PAR1 agonist peptides and the enzymatic activator of the receptor, thrombinin [Vouret-Craviari et al., 1992]. The reason for these divergent responses has become clearer in the light of discoveries indicating the existence of other thrombin receptors. In particular, the cloning and characterisation of PAR4 indicates that this receptor may control a late-phase of thrombin signalling corresponding to the latter part of two-phase responses often observed to thrombin [Kahan et al., 1992]. It is, therefore, important that thrombin and PAR1 peptide responses are reassessed in order to establish which members of the PAR family are responsible for the discrepant responses to the two agonists.

**PAR2 signalling**

The discovery of PAR2 underlined the potential importance of serine proteinase-mediated receptor activation. Tissue distribution of PAR2 has proved to be fairly wide, indicating that it may have a role in several physiological systems and classical pharmacological studies have supported PAR2 as having a role in many different tissue types [Macfarlane et al., 2001]. At a cellular level, there are still relatively few studies assessing the signalling pathways activated by PAR2, possibly due to relatively low expression levels of this receptor. PAR2 has been closely linked with both cell proliferation [Mirza et al., 1996; Akers et al., 2000; Fruniger et al., 2002; Gaca et al., 2002] and inflammation [Wakita et al., 1997; Vergnolle, 1999, 2000; Cocks and Moffat 2000; Vergnolle et al., 2001; Temkin et al., 2002; Ferral et al., 2003] in many tissue types and the signalling pathways thus far identified as being activated by PAR2 are typically associated with these proliferative and inflammatory cellular processes.

It has been established that PAR2 activation by trypsin or agonist peptide leads to increases in intracellular calcium levels together with the production of IP3 and DAG [Nysetedt et al., 1995; Santulli et al., 1995]. This signalling profile, typical of Gq/G11 coupled receptors acting via PLC isoforms, allows the receptor to influence a wide range of intracellular targets, similar to the case for PAR1. In addition, experiments with PAR2 expressed in the Xenopus oocyte system have indicated a degree of pertussis toxin sensitivity in PAR2 intracellular signalling events, indicating a role for Gq/11-dependent signalling [Schultheiss et al., 1997]. However relative to PAR1, little information is available regarding PAR2 coupling to G-proteins.

Nevertheless, PAR2 has been strongly linked to the activation of the MAP kinase family p42/44 MAP kinase (ERKs), JNK, and p38 MAP kinase. p42/44 MAP kinases are strongly linked with mitogenesis [Pages et al., 1993] and are ubiquitously expressed in mammalian tissues [Pearson et al., 2001]. This kinase cascade probably represents one of the most intensely studied kinase signalling pathways to date, and has a well-defined multi-step format that holds for all members of the family (see Fig. 1). The activation of p42/44 MAP kinase by PAR2 has been identified in several cell types [Belham et al., 1996; DeFea et al., 2000; Sabri et al., 2000; Gaca et al., 2002], and due to
the strong link between PAR2 and cell proliferation it was predictable that this important regulator of cell growth and differentiation would be activated by PAR2. The upstream coupling of PAR2 to these signalling pathways still requires further clarification to fully establish the G-proteins and other signalling intermediates involved. It has not, however, been established if PAR2 utilizes transactivation of growth factor signalling pathways to activate the MAP kinase, or any other, cascades. Interestingly, the activation of p42/44 MAP kinase by PAR2 has been suggested to require the internalization of the receptor [DeFea et al., 2000], indicating that the receptor may use alternative modes of signal transduction than merely directly activating G-protein intermediates. This work also provides an interesting adjunct to the idea of G-protein activation of the p42/44 MAP kinase cascade, as the necessity for receptor internalization may indicate an alternative mechanism for cascade activation. Possibly such a mechanism plays a role to aid in the appropriate subcellular localisation of the MAP kinase [Luttrell, 2002], together with maintaining the signal past the point of desensitization of the receptor.

Mitogenic signalling activated via PAR2 may prove to be an important area of study, due to the increasing evidence that identifies PAR2 expression as being increased in several tumor types [Darmoul et al., 2001; Ducroc et al., 2002; Jin et al., 2003], in addition to the receptor being linked to invasiveness in pancreatic cancer [Ikeda et al., 2003]. It has also been demonstrated that PAR2 can activate the important SH2-containing protein tyrosine phosphatase SHP-2 [Yu et al., 1997] that has previously been implicated in PAR1 activation of mitogenesis. Other tyrosine phosphorylations observed following activation of PAR1 have yet to be demonstrated for PAR2, despite these events relating to PAR1 activation, and transactivation, of p42/44 MAP kinase pathways. These differences possibly indicate fundamental differences in the signalling strategies employed by these two receptors. The use of tyrosine kinase inhibitors has been a feature of some pharmacological studies [Kawabata et al., 1999; Mule et al., 2002], but due to the fairly non-specific nature of these agents, the exact identity of the kinases involved remains conjectural.

**PAR2 ACTIVATION OF INFLAMMATORY SIGNALLING PATHWAYS**

The coupling of PAR2 with the SAP kinases JNK and p38 MAP kinase, together with the activation of the NFκB signalling pathway, is consistent with the overall view of PAR2 as being involved in inflammatory events (Fig. 2). Activation of c-fos, AP-1, CREB, and NFκB transcriptional activities by PAR2 [Yu et al., 1997; Kanke et al., 2001; Temkin et al., 2002] also indicates the receptor is capable of bringing about...
meaningful changes in the expression of important genes within cells. The SAP kinase and NFκB signalling pathways are well-documented regulators of inflammatory gene expression, and have been implicated in the control of cytokines such as IL-6, released from keratinocytes following PAR2 activation [Wakita et al., 1997], IL-8, and GM-CSF, amongst others. Activation of these inflammation-related signalling pathways by PAR2 has been demonstrated in diverse cell types such as cardiomyocytes [Sabri et al., 2000], coronary smooth muscle [Breitschneider et al., 1999], eosinophils [Temkin et al., 2002], and keratinocytes [Kanke et al., 2001], indicating that the activation of these signalling pathways is an important response to the activation of PAR2. Indeed, recent work points to a potentially important role for PAR2 in rheumatoid arthritis, a disease typified by chronic inflammation [Ferral et al., 2003].

The signalling of PAR2 to the transcription factor NFκB also requires further investigation. Previous study of this cascade has indicated that activation of the NFκB dimer may proceed via an IKK isoform-independent mechanism [Kanke et al., 2001]. This finding raises the possibility that PAR2 may activate a unique pathway for activation of this important inflammatory transcription factor. Such a pathway may enable PAR2 signals to NFκB to be isolated for treatment of inflammatory conditions in which PAR2 blockade is desirable.

PAR3 AND PAR4 ACTIVATED SIGNALLING

The investigation of signalling by PAR3 and PAR4 has received very little attention in the literature. As the roles these receptors play in physiological systems are elucidated, the intracellular signalling mechanisms associated with them shall become clearer. It has proved particularly difficult to establish the role played by PAR3 due to the initial lack of a functional synthetic agonist peptide ligand for the receptor [Ishihara et al., 1997; Nakanishi-Matsui et al., 2000; Sambrano et al., 2001]. Murine PAR3, in contrast to other members of the PAR family, does not appear to signal in response to its synthetic tethered activating peptide sequence, and unequivocal evidence for a response of human PAR3 to the synthetic PAR3-derived receptor-activating peptide has yet to be established. At present, it appears that PAR3 functions principally as a co-factor for the activation of PAR4 [Nakanishi-Matsui et al., 2000; Sambrano et al., 2001]. However, the expression of PAR3 and PAR4 in different tissues, either alone or in combination suggests that PAR3 may play a functional role that has yet to be determined.

An early study of PAR4 identified that activation of this receptor can lead to increases in the level of intracellular calcium in human astrocytoma cells [Kaufmann et al., 2000]. More recently the suggestion has been made that in cardiomyocytes the PAR4 specific agonist peptide AYPGFK can activate p38 MAP kinase, but shows limited ability to activate PLC and p42/44 MAP kinase [Sabri et al., 2003]. Interestingly, this study also contends that in these cells the activation of c-Src by thrombin is a PAR4 mediated event, and that the activation of p38 MAP kinase is via transactivation of the epidermal growth factor receptor (EGFR) signalling intermediates.

CONCLUDING REMARKS

As noted earlier, the scope of the studies into the intracellular signalling of the PAR family as a whole has been fairly limited. However, as the pace of research into this novel family of receptors increases as it has in recent years, it is likely that the methods employed by these receptors to signal within cells will come under greater scrutiny. This should lead to better understanding of the PARs and advance their potential as therapeutic targets in a wide range of disease states.

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REFERENCES


PAR-MEDIATED SIGNAL TRANSDUCTION


