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REVIEW

Proteinases, their receptors and inflammatory signalling: the Oxford South Parks Road connection*

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Nomenclature: In this article drug/molecular target nomenclature conforms to BJP's Guide to Receptors and Channels (Alexander *et al.*, 2013a,b,c); amino acids are abbreviated by their one-letter codes, for example, O, ornithine; S, serine; Y, tyrosine.

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In keeping with the aim of the Paton Memorial Lecture to 'facilitate the historical study of pharmacology', this overview, which is my distinct honour to write, represents a 'Janus-like' personal perspective looking both backwards and forwards at the birth and growth of 'receptor molecular pharmacology' with special relevance to inflammatory diseases. The overview begins in the Oxford Department of Pharmacology in the mid-1960s and then goes on to provide a current perspective of signalling by proteinases. Looking backwards, the synopsis describes the fruitful Oxford Pharmacology Department infrastructure that Bill Paton generated in keeping with the blueprint begun by his predecessor, J H Burn. Looking forwards, the overview illustrates the legacy of that environment in generating some of the first receptor ligand-binding data and providing the inspiration and vision for those like me who were training in the department at the same time. With apologies, I mention only in passing a number of individuals who benefitted from the 'South Parks Road connection' using myself as one of the 'outcome study' examples. It is also by looking forward that I can meet the complementary aim of summarizing the lecture presented at a 'BPS 2014 Focused Meeting on Cell Signalling' to provide an overview of the role of proteinases and their signalling mechanisms in the setting of inflammation.

LINKED ARTICLES

This article is part of a themed section on 5th BPS Focused Meeting on Cell Signalling. To view the other articles in this section visit http://dx.doi.org/10.1111/bph.2015.172.issue-13

Abbreviations

DU145, prostate cancer-derived cell obtained from a dura matter metastasis; KLK, kallikrein-related peptidase; mCherry, red fluorescent protein derived from a protein isolated from *Discosoma* sp.; MOG, myelin oligodendrocyte glycopeptide; MS, multiple sclerosis; NE, neutrophil elastase; PAR, proteinase-activated receptor; PAR-AP, PAR-activating peptide; PC3, prostate cancer-derived cell obtained from a bone metastasis; TL, tethered ligand; TRAP, thrombin receptor-activating peptide; YFP, yellow fluorescent protein

Tables of Links

Targets	
GPCRs[a]	**Enzymes**[c]
AT_1 receptor	Adenylyl cyclase
Glucagon receptor	Chymotrypsin
MC_2 (ACTH) receptor	Kallikrein-related peptidase (KLK) 6
PAR	Neutrophil elastase (NE)
Catalytic receptors[b]	MMP1
EGFR	p38MAPK
Insulin receptor	p42MAPK
PDGFR	p44MAPK
	Proteinase 3

Ligands
ADP
CGRP
EGF
Insulin
Substance P
Thrombin

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 ([a,b,c]Alexander *et al.*, 2013a,b,c).

Introduction

The South Parks Road environment, 1964–1968, and the 'butterfly effect'

On entering the Oxford Department of Pharmacology in 1964 as a rather naïve, newly minted Canadian Rhodes Scholar with a background in chemistry, I was only dimly aware of the rich pharmacology-focused environment I had joined. The department shaped by W D M Paton, continuing in the directions set by his predecessor, J H Burn (Vane, 1982), accurately reflected Paton's own view of pharmacology in terms of its close relationship with chemistry and its ability to progress 'from the molecular level to the whole man' (Paton, 1986). Fortunately for me, Paton at that time also believed that the discipline was 'not yet too sophisticated technically, and still young and fresh enough for the simpleminded to contribute' (Paton, 1986). Clearly I could qualify, and I was immediately sold on the idea of pharmacology as a career. However, I had no idea either of the impact that the relatively short time in the department would have on me or the long road ahead that would continually be influenced by my mentors who 'took me on' in the Oxford Department.

Fortunately for me, I found that, including Paton, the department was inhabited by quite a number of 'chaos theory butterflies' (Figure 1) who with the slightest flap of their wings were able to trigger a virtual hurricane of pharmacology progress for the future.[1] I was only one among many who were 'launched from the Department' during the short time frame of my 'residency' there (1964–1968). Thus, the department members who influenced many trainees like myself had a long-lasting impact on the growth of pharmacology in unpredictable directions.

Everything was there for me in the department: (i) the technical support of O B Saxby; (ii) the chemical and biochemical expertise of E W Gill and H K F Blaschko; (iii) the peptide chemistry of D B Hope; (iv) the receptor insights provided by H P Rang and W D M Paton; and (v) the in-depth understanding of the physiology and pharmacology of smooth muscle provided by E Bulbring and A F Brading. Of those in the department at that time, I have singled out the main individuals (Figure 1) who most likely unknowingly have had a major impact on my current work dealing with proteinases and their signalling mechanisms that are involved in inflammatory processes. In sum, the department in the mid-1960s fostered by Paton provided a superb infrastructure that served as the 'butterfly' to generate an enormous impact on the future development of the discipline of molecular pharmacology. An overview of our own work on proteinase-mediated signalling, which clearly has its roots in my experience in Oxford, is provided in the following paragraphs. The text will outline the 'hormone-like' role that proteinases can play in the setting of inflammation in part by activating the so-called proteinase-activated receptors or 'PARs'. The synopsis focuses primarily on the impact of my Oxford experience on our work linking proteinase-mediated signalling to inflammation. For more comprehensive reviews, the reader is referred elsewhere (Coughlin, 2005; Ramachandran and Hollenberg, 2008; Adams *et al.*, 2011; Ramachandran *et al.*, 2012; Alexander *et al.*, 2013; Hollenberg, 2014; Hollenberg *et al.*, 2014; Zhao *et al.*, 2014).

Proteinase-mediated signalling

Since more than 2% of the human genome is now known to code for either proteinases (colloquially termed proteases) or their inhibitors (Puente *et al.*, 2005), it is given that these

[1]In chaos theory, the 'butterfly effect' is the sensitive dependence on initial conditions, where a small change at one place in a non-linear system can result in large differences to a later state (Wikipedia, 2014a). The effect derives its name from the theoretical example of a hurricane's formation being contingent on whether or not a distant butterfly had previously flapped its wings.

Figure 1

The 'mentor butterflies' of the Oxford Department of Pharmacology as of 1964–1968. The photos identify the mentors in the Oxford Department of Pharmacology between 1964 and 1968 who provided key insights in essential areas (listed along with their names) that have underpinned the work performed in my laboratory to characterize the PARs and their inflammatory actions. Photos left to right; top to bottom: (i) Derek Hope, with me on his left: grounding in peptide chemistry. (ii) Professor Bill Paton: fundamentals of the rate theory of receptor function, provision of overall departmental infrastructure. (iii) Hugh Blaschko: basics of biochemical pharmacology, enzyme isolation and kinetics, and hormone biosynthesis. (iv) Humprey Rang: key concepts of receptor molecular pharmacology, receptor ligand binding. (v) Edith Bulbring: details of smooth muscle pharmacology and function. [Photos adapted from private and public databases (Google) and from (i) Rang & Lord Perry (1996) with permission; Wikipedia (2014b) and (ii) Blaschko (1980) with permission.]

enzymes will be found to serve multiple biological roles. Thus, it comes as no surprise that in addition to their 'classical roles' as digestive protein-degrading enzymes, proteinases can now be categorized as hormone-like mediators that regulate tissue function by both receptor and non-receptor mechanisms (Figure 2). Not only was it appreciated by the mid-1960s when I was conducting my DPhil project that peptide hormones were generated from precursor 'pro-hormones' and metabolized by proteolytic processing (Steiner *et al.*, 1967; Steiner, 2011; Chretien, 2012), but it was also known that enzymes such as trypsin and chymotrypsin had hormone-like metabolic–anabolic actions akin to the effects of insulin (Rieser and Rieser, 1964; Rieser, 1967; Kono and Barham, 1971). This 'insulin-like' action of trypsin on adipocytes can now be seen to result from the cleavage of the extracellular α-subunit of the insulin receptor so as to abolish its inhibitory control of receptor function (Shoelson *et al.*, 1988). At higher concentrations, trypsin can be seen to 'disarm' the insulin receptor by removing the insulin-binding domain, thereby preventing signal transduction (Cuatrecasas, 1969; 1971). By the 1970s it was also clear that thrombin and trypsin could stimulate mitogenesis in cultured cells by a receptor-like mechanism, thus mimicking the actions of polypeptide growth factors such as insulin and EGF (Burger, 1970; Sefton and Rubin, 1970; Chen and Buchanan, 1975; Carney and Cunningham, 1977; 1978). However, the mechanisms whereby these proteinases caused their mitogenic actions were a mystery at the time. As outlined in the following paragraphs, it was not until a decade or so later that a receptor mechanism for these hormone-like actions of proteinases was discovered. That discovery resulted from the search for the 'thrombin receptor' responsible for stimulating (i) human platelet aggregation and (ii) mitogenesis in cultured hamster cells. Surprisingly, it turned out that a GPCR (PAR) is responsible for these actions of thrombin (Rasmussen *et al.*, 1991; Vu *et al.*, 1991). Although there are multiple mechanisms whereby proteinases can signal to cells, as outlined in Figure 2A, the main focus of this overview will be on the so-called PARs for which a unique mechanism of proteolytic activation was discovered by the Coughlin laboratory (Vu *et al.*, 1991). The PAR and non-PAR mechanisms of proteinase signalling (Figure 2A and B) can be seen as com-

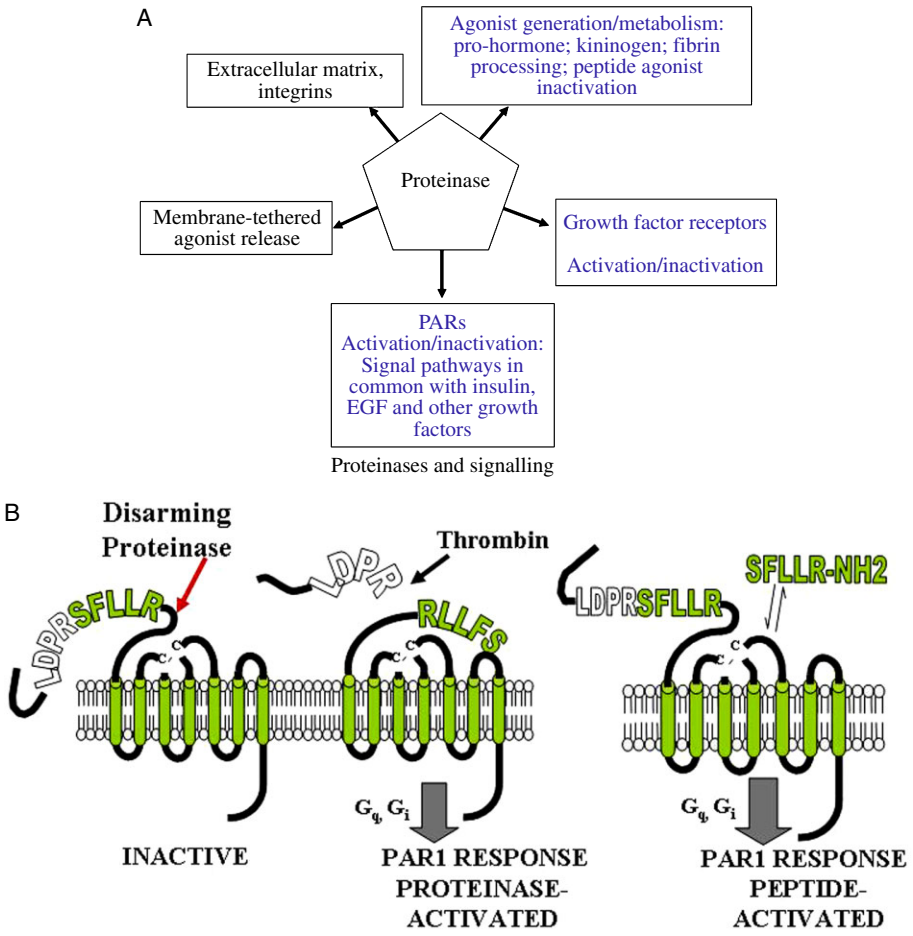

Figure 2

Signalling by proteolytic enzymes and the mechanism of activation of PARs. (A) (Upper) The diagram shows five distinct ways by which proteinases can trigger cell signalling, ranging from the generation or degradation of agonist peptides (top) to the activation of PARs (bottom). (B) (Lower) The scheme shows the proteolytic activation of human PAR1 (non-activated receptor on left) either by the unmasking of a receptor TL (middle: SFLLR--- for PAR1) or by a synthetic peptide with a sequence in common with the revealed TL (right: SFLLR-NH_2).

plementary mechanisms whereby tissue-derived proteinases can play key roles in physiologically important processes.

Signalling by thrombin and discovery of the four-member PAR GPCR family members

As already alluded to, two laboratories independently cloned a receptor responsible for the ability of thrombin to stimulate cell replication in cultured hamster cells (Rasmussen *et al.*, 1991) and to regulate human platelet function (Vu *et al.*, 1991). A key feature of the cloned GPCR observed in both laboratories was its ability to mobilize intracellular calcium when activated by thrombin, presumably by triggering Gq (Rasmussen *et al.*, 1991; Vu *et al.*, 1991). Thrombin had already also been observed to inhibit AC most likely by coupling directly with Gi (Vouret-Craviari *et al.*, 1992). However, an indirect activation of a Gi-coupled receptor via the release of a second agonist (e.g. via ADP release in platelets) is also possible. Thus, early on, it was realized that thrombin via its cloned receptor (now termed PAR1, with its gene designated, *F2R*) could signal by coupling to multiple G-proteins, with a possible preference for activating Gq. The unique mechanism discovered for activating this unusual G-protein coupled 'thrombin' receptor (Vu *et al.*, 1991) involves the proteolytic unmasking of a cryptic N-terminal cell-attached receptor sequence that folds back acting as a 'tethered ligand' (TL) to trigger signalling (Figure 2B, middle panel). Once activated, the receptor targets other effectors both in the plane of the membrane (e.g. within seconds to minutes: Gq) and upon internalization (minutes to 10 s of minutes: formation of endosomal β-arrestin signalosomes: Defea, 2008; Grimsey *et al.*, 2014). The down-regulation of PAR signalling involves three distinct processes including desensitization, internalization and lysosomal degradation. Thus, as opposed to other GPCRs that can recycle to the cell surface for re-activation, it is believed that PAR signalling follows the 'Kleenex® principle' (Kimberly-Clark Worldwide Inc., Pleasant Prairie, WI, USA; Brampton, ON, Canada) – use only once – then dispose (Grimsey *et al.*, 2011). Of great significance, the Coughlin laboratory discovered that synthetic peptides with sequences corresponding to the proteolytically exposed N-terminal 'TL' domain were found to stimulate receptor signalling without the need for proteolytic activation (Vu *et al.*, 1991) (Figure 2B,

Table 1

The human PAR family, their canonical and non-canonical proteolytically unmasked tethered ligands and their non-biased and biased peptide agonists[a]

Canonical and non-canonical PAR cleavage sites and tethered ligand sequences				
Receptor/gene designation	**Canonical tethered ligand (human)**	**Receptor-selective PAR-APs**	**Non-canonical tethered ligands**	**Biased PAR-APs**
PAR1/F2R	--//SFLLRN--	TFLLR-NH_2	MMP1: --//PRSFLLRN-- PR3: --//TLDPRSF-- APC: -//NPNDKYEPF— NE: -//RNPNDKYEPF	PRSFLLRN; NPNDKYEPF; RNPNDKYEPF YFLLRN
PAR2/F2RL1	--//SLIGKV--	SLIGRL-NH_2; 2-furoyl-LIGRL-NH_2	NE: --//VLTGKL--	SLAAAA-NH_2
PAR3/F2RL2	--//TFRGAP--	TL-derived peptides activate PARs 1 and 2	Not known	Not known
PAR4/F2RL3	--//GYPGQV--	AYPGQV-NH_2; AYPGKV-NH_2	Not known	Not known

[a]The 'canonical' PAR 'tethered ligands' resulting from cleavage activation of the human PARs (cleavage site shown as: //) correspond to the sequences of the receptor-selective PAR-APs, for example, SFLLRN – as shown in Figure 2B. The sequences of synthetic receptor-selective 'canonical' PAR-APs based on the thrombin (PARs 1 and 4) or trypsin (PAR2)-unmasked tethered ligands are shown along with 'non-canonical' tethered ligands unmasked (//) by NE (PAR 2), MMP1 (PAR1) or activated protein-C (APC: PAR1) as outlined in the text. Further, biased synthetic PAR-APs derived from the 'non-canonical' enzyme-unmasked tethered ligands are shown in the last column on the right. Adapted from Hollenberg *et al.* (2014) with permission.

right-hand panel). Thus on its own, the sequence, SFLLRNPNDKYEPF, can mimic the action of thrombin on human platelets (Vu *et al.*, 1991). However, it was quickly realized from studies of the actions of this 'thrombin receptor-activating peptide' (so-called TRAP) on rabbit and rat platelets that there was a thrombin receptor on rodent platelets that could not be activated by the human TRAP (Kinlough-Rathbone *et al.*, 1993). That said, the TRAP peptide was able to activate rodent thrombin receptor responses in tissues other than platelets, verifying that the rodent platelet receptor differed from the one in other sites. With the insight gained from my Oxford Department work on the actions of oxytocin and vasopressin and from my grounding in receptor structure–activity studies, it was clear to me that a receptor different from the thrombin receptor cloned by Vu and colleagues in 1991 had to be present on rodent platelets. As a result, this 'echo' of my work carried out in Oxford on the neurohypophysial peptide hormones, which have overlapping receptor selectivity, led us to use the same structure–activity principles whereby Ahlquist (1948) identified α- and β-adrenoceptors to evaluate the thrombin receptor: we sought to identify 'thrombin receptor subtypes' with the use of a series of distinct receptor-activating agonists. Based on my 'peptide' legacy from Derek Hope, we thus went ahead to synthesize a small number of peptide thrombin receptor agonist analogues based on the 'SFLLRN---' sequence and proceeded to determine their relative potencies for regulating tension in vascular and gastric smooth muscle tissue preparations (Hollenberg *et al.*, 1993; Al-Ani *et al.*, 1995). This choice of smooth muscle targets was prompted by my previous interactions in Oxford with the Bulbring and Brading laboratories where work drew my attention to the value of using smooth muscle responses for bioassays as already described in the literature. Unequivocally, we were able to show pharmacologically that there are distinct receptor subtypes for PAR-activating peptides (PAR-APs) in the vascular endothelium and in gastric smooth muscle (Hollenberg *et al.*, 1993). However, our cloning efforts at the time were not able to identify the 'second' PAR-AP receptor in vascular tissues that differs from the one in human platelets. That vascular receptor turned out to be PAR2/*F2RL1* as described in the following paragraph.

The discovery of that vascular receptor came from a search of a genomic library for a substance K receptor (Nystedt *et al.*, 1994), which identified a 'new' receptor (now termed, PAR2/*F2RL1*) that had sequence homology with PAR1, which is present on human platelets. However, the new PAR was activated by trypsin and not thrombin. Shortly after the cloning of murine PAR2, human PAR2/*F2RL1* was also cloned (Nystedt *et al.*, 1995; Bohm *et al.*, 1996). We found that trypsin revealed the TL sequence of mouse PAR2 (SLIGRL-NH_2), which did not activate the 'thrombin receptor/PAR1' but did, nonetheless stimulate endothelium-dependent vasorelaxation. We were thus able to conclude that endothelial PAR2 is responsible for the vasorelaxant action of trypsin and some of the PAR-APs that we had studied (Al-Ani *et al.*, 1995). The cloning of PARs 1 and 2 paved the way for the cloning of the other two members of the PAR family, namely PAR3/*F2RL2* (Connolly *et al.*, 1996; Ishihara *et al.*, 1997) and PAR4/*F2RL3* (Kahn *et al.*, 1998; Xu *et al.*, 1998). All of the PARs have in their N-terminal sequences a principal serine proteinase-targeted arginine at which cleavage exposes a distinct TL for each receptor as summarized in Table 1. Thrombin is able to activate PARs 1 and 4, both of which are present on human platelets, whereas trypsin activates PAR2 now known to be present in vascular endothelial cells among other sites including intestinal epithelial cells (Nystedt *et al.*, 1994; 1995; Bohm *et al.*, 1996). Unmasking of such TLs in PARs 1 and 4 by thrombin and in PAR2 by trypsin stimulates cell signalling that involves a number of G-proteins (Gq, Gi, G12/13). Further, the syn-

thetic peptides based on the revealed TL sequences can also stimulate comparable signalling via the 'partner' G-proteins. As outlined in Table 1, it has been possible to synthesize PAR-selective activating peptides to evaluate the impact of signalling by PARs 1, 2 and 4 in a variety of cultured cell and *in vivo* contexts without the need to use proteinases to activate the receptors. PAR3 appears to function as a 'cofactor' for activation of PAR4 (Nakanishi-Matsui *et al.*, 2000) and peptides derived from its TL sequence are able to activate both PARs 1 and 2 (Hansen *et al.*, 2004). In certain circumstances, PAR3 is reported to signal on its own (Ostrowska and Reiser, 2008), but its general role in regulating tissue function remains to be fully elucidated.

To sum up, the ability of serine proteinases to regulate tissues can now be seen to include activation of PARs by a 'TL mechanism' in addition to the other signalling processes illustrated in Figure 2A. The unusual features of the PARs are now well appreciated (Figure 2B) in terms of (i) their unique mechanism of proteolytic activation, involving the exposure of a TL (middle panel, Figure 2B); (ii) their ability to be activated selectively by receptor-specific synthetic-activating peptides based on the sequences of the revealed TL (right panel, Figure 2B; Table 1); and (iii) their susceptibility to be activated or inactivated/disarmed (left-hand panel, Figure 2B, red arrow) selectively by a variety of serine and other proteinases. For instance, thrombin activates PARs 1 and 4, but not PAR2; trypsin at low concentrations can activate PAR2 and PAR4 but disarms PAR1 by removing the TL (Kawabata *et al.*, 1999); and *Pseudomonas* elastase disarms trypsin-mediated activation of PAR2 (Dulon *et al.*, 2005). It is important to recognize that a proteinase that cleaves C-terminal to the TL domain sequence shown by the red arrow in the left panel of Figure 2A (. . . LDPRSFLLR . . .) will disarm the PAR to prevent its activation by an enzyme (e.g. thrombin) that acts via the exposed TL. Thus, enzymatically, the PARs can be seen to have their circulating proteinase 'agonists' that unmask the TL as well as circulating 'antagonist' proteinases that silence the receptors by cleaving downstream of the TL and removing it from the receptor. However, the unanswered questions that remain are (i) Do the synthetic TL PAR-APs stimulate the *identical* signals triggered by the proteinase-revealed TLs? and (ii) Which endogenous proteinases regulate PAR function *in vivo*? Work aimed at answering these questions has revealed the ability of the PARs to signal in a 'biased' way as illustrated for GPCR in general in Figure 3. This 'functional selectivity' of PAR signalling can be stimulated either by 'biased agonist receptor-activating peptides' (Table 1, column 4) or by proteinases that cleave the N-terminal PAR sequences at sites that differ from the 'classical' TL domain.

The mobile receptor paradigm and functional selectivity of PAR signalling

Multiplicity of receptor signalling

By the mid-1970s, it was appreciated that many different receptors, for example, the ones for ACTH and glucagon (now known to be GPCR) as well as the ones for insulin and EGF (now termed 'growth factor receptors'), could signal via common biochemical pathways (e.g. AC activation by multiple GPCRs or common anabolic enzyme pathways triggered by insulin and EGF). Further, it was known that a single receptor could signal via more than one pathway (e.g. activation of calcium signalling as well as inhibition of AC by the angiotensin AT_1 receptor). This multiplicity of signalling by an individual receptor was rationalized in terms of a 'mobile' or 'floating' receptor model (de Haën, 1976; Jacobs and Cuatrecasas, 1976). In this model, an individual receptor was seen as potentially interacting with multiple 'effectors' in the plane of the membrane, and further multiple receptors in the same membrane environment were postulated to be able to regulate the same effector. For 'growth factor' receptors (e.g. for insulin, EGF and PDGF), this 'modular' model encompasses multiple common signalling pathways involving SH2–SH3 domain interactions between the tyrosine-phosphorylated receptors and their multiple SH2–SH3 domain-containing effectors (Jin and Pawson, 2012). For GPCRs, this model enables 'selective' signalling via different G-proteins with which an individual receptor can couple as outlined in Figure 3. This type of signalling has been termed functional selectivity or 'biased signalling' (Kenakin, 2012; 2013). PARs are no exception to this paradigm of pathway-selective signalling (Hollenberg *et al.*, 2014; Zhao *et al.*, 2014).

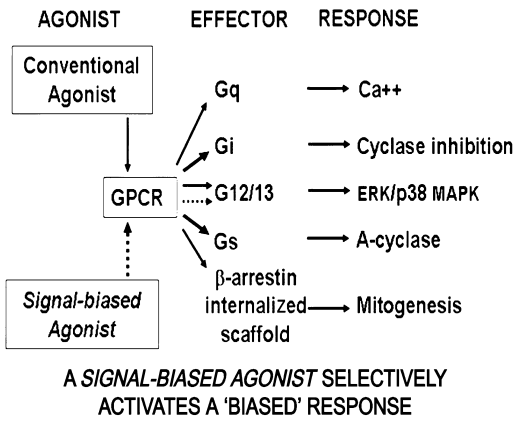

Figure 3

Biased, functionally selective signalling. The scheme, in keeping with the 'floating' or 'mobile receptor' model outlined in the text, shows that a 'conventional' agonist can drive a GPCR to interact with multiple 'G-protein effectors' in the plane of the membrane (e.g. Gq, Gi, G12/13). However, a 'biased agonist' is shown (dashed lines) to promote a functionally selective interaction of the receptor with only one (e.g. G12/13) of several potential G-protein effectors. [Adapted from Hollenberg *et al.* (2014) with permission.]

Early on, from structure–activity studies with the PAR-APs, it was realized that proteolytic signalling (e.g. by thrombin for PAR1) was not completely recapitulated by the receptor-activating peptides. As an example, the mitogenic MAPK-stimulating action of thrombin in hamster fibroblasts was not matched by the action of the receptor peptide agonist (Vouret-Craviari *et al.*, 1992; 1993). Further, studies of PAR1 with mutations in its extracellular domains showed that the receptor sites for binding the PAR1-activating peptides (most mutations affected PAR-AP-induced responses) differed substantially from the sites involved in PAR1 activation by the thrombin-revealed TL (responses largely unaffected by mutation) (Blackhart *et al.*, 2000). Our own work

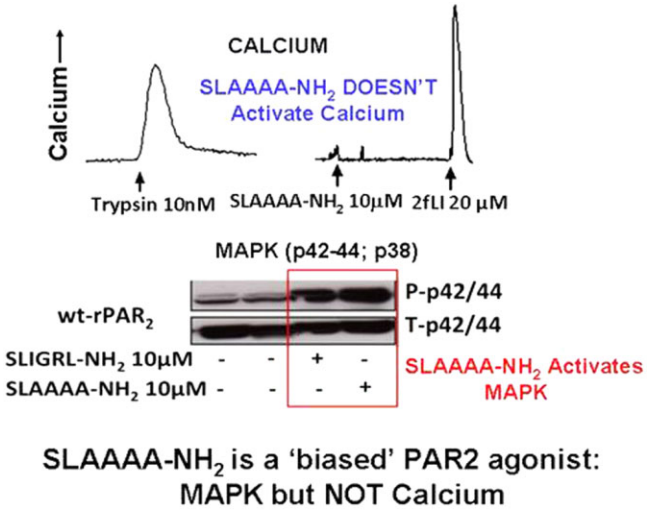

Figure 4

Biased PAR2 signalling by the receptor-activating peptide, SLAAAA-NH_2: activates MAPKinase but not calcium transients. Upper tracings: trypsin activation of PAR2 (upper, left tracing) causes a PAR2-dependent increase in intracellular calcium (upward deflection of tracing). However, the PAR2-activating peptide, SLAAAA-NH_2 with multiple alanine substitutions, does not trigger calcium signalling (upper right tracing) like the full PAR2 peptide agonist, 2-furoyl-LIGRLO-NH_2, subsequently added to the cells (right-hand portion of upper right tracing). Lower, Western blots: However, the increase in phospho-MAPK (Western blot signal, red-outlined box) caused by activation of PAR2 by SLAAAA-NH_2 is equivalent to the signal generated by the non-biased PAR2 peptide agonist, SLIGRL-NH_2. Thus, SLAAAA-NH_2 is a 'biased agonist' for PAR2. [Adapted from Hollenberg *et al.* (2014) with permission.]

with PAR2 then showed that mutations in the extracellular PAR2 loop-2 domain (wild-type 'EE' mutated to 'RR' in this extracellular loop) markedly affected calcium signalling by the PAR2-activating peptide, SLIGRL-amide, but had little or no effect on calcium signalling by the trypsin-revealed TL (Al-Ani *et al.*, 2002). Thus for PAR2 as well as for PAR1, the signalling interactions of the proteolytically unmasked TL sequences can be seen to differ from signalling triggered by the synthetic receptor-activating peptides. To our surprise, we found that an alanine-substituted synthetic peptide based on the proteolytically revealed PAR2 TL (SLAAAA-amide vs. SLIGRL-amide) failed to activate PAR2 calcium signalling (Figure 4, upper panel), but was able to activate PAR2 MAP-Kinase signalling (table 2 in Al-Ani *et al.*, 2004; Figure 4, lower panel; Ramachandran *et al.*, 2009). Thus, we concluded that for both PARs 1 and 2, the change in receptor conformation caused by the exposed TL differs from changes stimulated by the synthetic PAR-APs. Further, we had identified a PAR2 'biased agonist' (SLAAAA-amide) that can selectively trigger MAPK activation but not calcium signalling. Previous work with a PAR1 agonist, YFLLRNP, pointed to its ability to act as a 'biased' partial agonist for PAR1 and able to stimulate human platelet shape change but not aggregation (Rasmussen *et al.*, 1993). Thus, it has become clear that like other GPCRs, the PARs are capable of functional selectivity or biased signalling as shown schematically in Figures 3 and 4 (Kenakin and Miller, 2010; Kenakin, 2011; 2012; 2013). What was not determined was whether activation of the PARs by endogenous proteinases might also be able to stimulate the receptors in a 'biased way' like the synthetic peptide agonists. Further, the mechanisms that could account for biased PAR signalling had not been determined.

Proteinase-triggered biased PAR signalling

When we found that the PAR2-activating peptide, SLAAAA-amide can stimulate MAPK signalling but not calcium signalling, we hypothesized further that proteolytic activation of PAR2 by enzymes that do not target the known cleavage activation sequence might also signal in a biased way. Since tissue inflammation, in which we knew PAR2 participates, involves the influx of neutrophils, we evaluated the ability of neutrophil proteinases (elastase, proteinase-3, cathepsin-G) to regulate PAR2 signalling. Indeed, in keeping with our observations with pseudomonas elastase (Dulon *et al.*, 2005), all of the three neutrophil enzymes were able to disarm PAR2 (e.g. Figure 2B, 'disarming proteinase'), thus preventing trypsin from stimulating PAR2 calcium signalling (Figure 5A, left; and see figure 1 in Ramachandran *et al.*, 2011). However, neutrophil elastase, while 'disarming' PAR2 calcium signalling by trypsin (Figure 5A, left panel), caused an activation of MAPK (Figure 5B,C, right upper and lower panel). In contrast, neither cathepsin-G nor proteinase-3 activated PAR2 MAPK signalling (not shown; Ramachandran *et al.*, 2011). This 'biased PAR2 signalling' stimulated by neutrophil elastase is due to the proteolytic unmasking of a distinct receptor-activating TL that is downstream from the one exposed by trypsin (Table 1, column 4) (Ramachandran *et al.*, 2011).

In a comparable way, neutrophil elastase and proteinase-3 also disarm thrombin-stimulated PAR1 signalling, but both enzymes can activate PAR1 MAPK signalling (Mihara *et al.*, 2013). The two proteinases do so by cleaving and unmasking distinct PAR1 'TL sequences' (Table 1, column 4). Both activated protein-C and MMP are also able to cleave the N-terminus of PAR1 to stimulate biased signalling (Boire *et al.*, 2005; Trivedi *et al.*, 2009; Mosnier *et al.*, 2012; Schuepbach *et al.*, 2012). Thus, the N-terminal domains of PARs 1 and 2 contain different cryptic sequences, which can drive signalling in distinct biased ways, depending on the activating proteinases (Table 1). Whether PAR4 also possesses different cryptic TL sequences remains to be evaluated. This versatility of signalling by the PARs provides a great diversity by which these receptors can respond in an inflammatory environment in which different proteinases are active.

Imaging PAR activation, mobility, internalization and signalling

As illustrated by the scheme in Figure 3, the ability of an individual receptor to activate multiple signalling pathways as described by the mobile or floating receptor model put forward some time ago (de Haën, 1976; Jacobs and Cuatrecasas, 1976) depends on its diffusion in the plane of the membrane to interact with multiple effectors (e.g. different G-proteins), and in the case of GPCRs like PAR2, to interact with β-arrestin, forming an internalized G-protein-independent signalling scaffold (Defea, 2008). We therefore set the goal of visualizing the PARs in the course of enzyme and peptide-stimulated signalling.

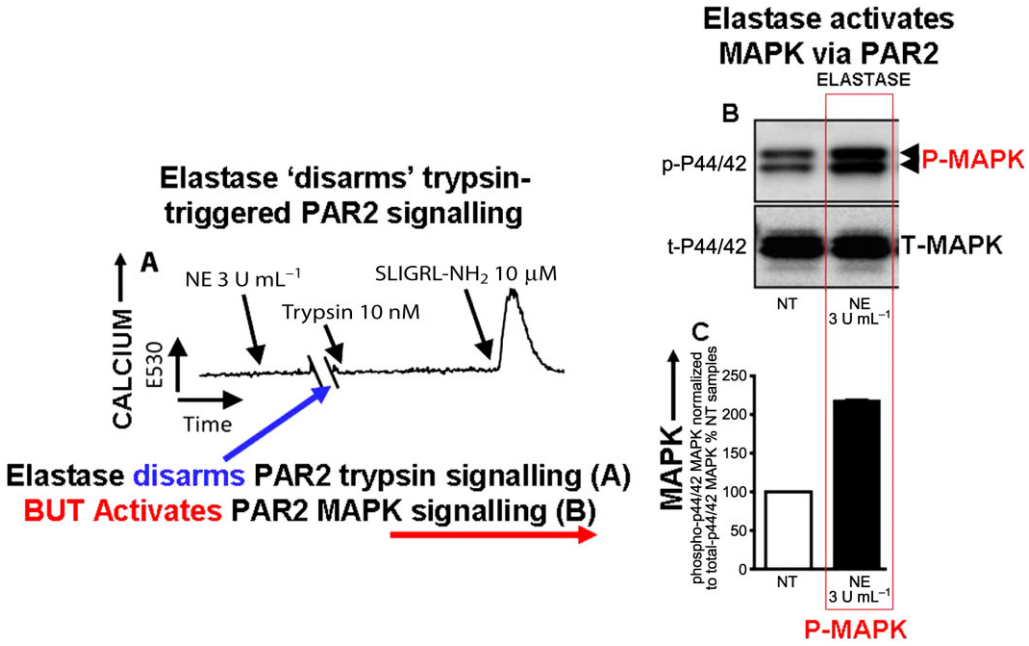

Figure 5

Biased PAR2 signalling by neutrophil elastase: activating MAPK, not calcium. Left tracings: neutrophil elastase disarms/silences trypsin-stimulated calcium signalling (but *not* peptide-mediated calcium signalling: E530, upward deflection) without causing a calcium signal on its own (A). Right Western blots: nonetheless elastase activates MAPK [red box outline on right: P-MAPK, arrows, upper right (B); filled histogram, lower right (C)]. [Adapted from Hollenberg *et al.* (2014) with permission.]

Following the footsteps of the work of Paton and Rang (1965) who measured the binding of radiolabelled atropine to its muscarinic receptor, we first developed a PAR2 ligand-binding assay using a tritium-labelled PAR2 peptide agonist (Al-Ani *et al.*, 1999). We then went on to deal with questions that could not be answered for receptors being studied in the mid-1960s: (i) Where is the receptor in the cell? and (ii) How does PAR activation affect its mobility and internalization in the course of triggering a signal?

In our first steps to answer these questions, we were able to visualize PAR2 internalization from the cell surface using a receptor-targeted antibody (see figure 1 in Al-Ani *et al.*, 1999). However, this approach was not optimal to visualize the mobility of the receptor in 'real time'. Therefore, to follow the dynamics of the activation of the PARs, we have generated receptors tagged C-terminally with yellow fluorescent protein (YFP) that can be used in cell expression systems to monitor both internalization and interactions with other effectors. Internalization is visualized by fluorescence imaging (see figure 9 in Ramachandran *et al.*, 2011) and receptor–YFP/effector–luciferase interactions are measured in real time by measuring BRET between the interacting partners (Ramachandran *et al.*, 2011). Further, to monitor the activation process of receptor cleavage, we have now prepared 'dually tagged' PARs. As shown for PAR1 in Figure 6 (upper), when the receptor is intact, the 'red' mCherry fluorogen attached to the receptor N-terminus adds to the 'green' fluorescence of the YFP fluorogen on the C-terminus so that the receptor appears in large part 'yellow'. Proteolytic activation of PAR1 by thrombin removes the N-terminal mCherry fluorogen so that the 'activated' receptor then appears green due to the remaining C-terminal YFP tag (Figure 6, upper). The activation process is visualized in intact cells as shown in the lower panel of Figure 6. Upon thrombin exposure, the receptor internalizes (green dots, Figure 6B, lower panel), with some cleaved/activated receptor remaining at the cell surface. The released N-terminal mCherry fragment is also internalized (red dots, Figure 6B, lower panel). Of note, even in the non-activated cell, we observe some 'background' green/cleaved receptor (e.g. Figure 6A, lower panel), suggesting that the expressed receptor is constitutively exposed to cell-secreted PAR-cleaving enzymes in the culture medium. In contrast with proteinase-mediated activation, the dynamics of receptor activation with a PAR-AP (TFLLR-NH_2: Figure 6C, lower panel) show that yellow intact receptor becomes internalized. Further, activation of the receptor with the PAR1-activating peptide also leads to the generation of internalized green receptor along with internalized red receptor N-terminal fragments (Figure 6C, lower panel). Thus, proteinase-*independent* receptor activation on its own, triggered by PAR-APs, appears to liberate receptor-cleaving proteinases in proximity to the activated cell. This issue, which has yet to be explored in any depth, illustrates the complexity of PAR activation in a host cell and suggested to us that there may be an 'autocrine' loop whereby cell stimulation via PARs or other receptors may release PAR-regulating proteinases as discussed briefly below.

Visualizing the distinct dynamics of proteinase-stimulated biased PAR signalling

Having developed an imaging approach to monitor PAR cleavage and activation, we next compared the dynamics of PAR1

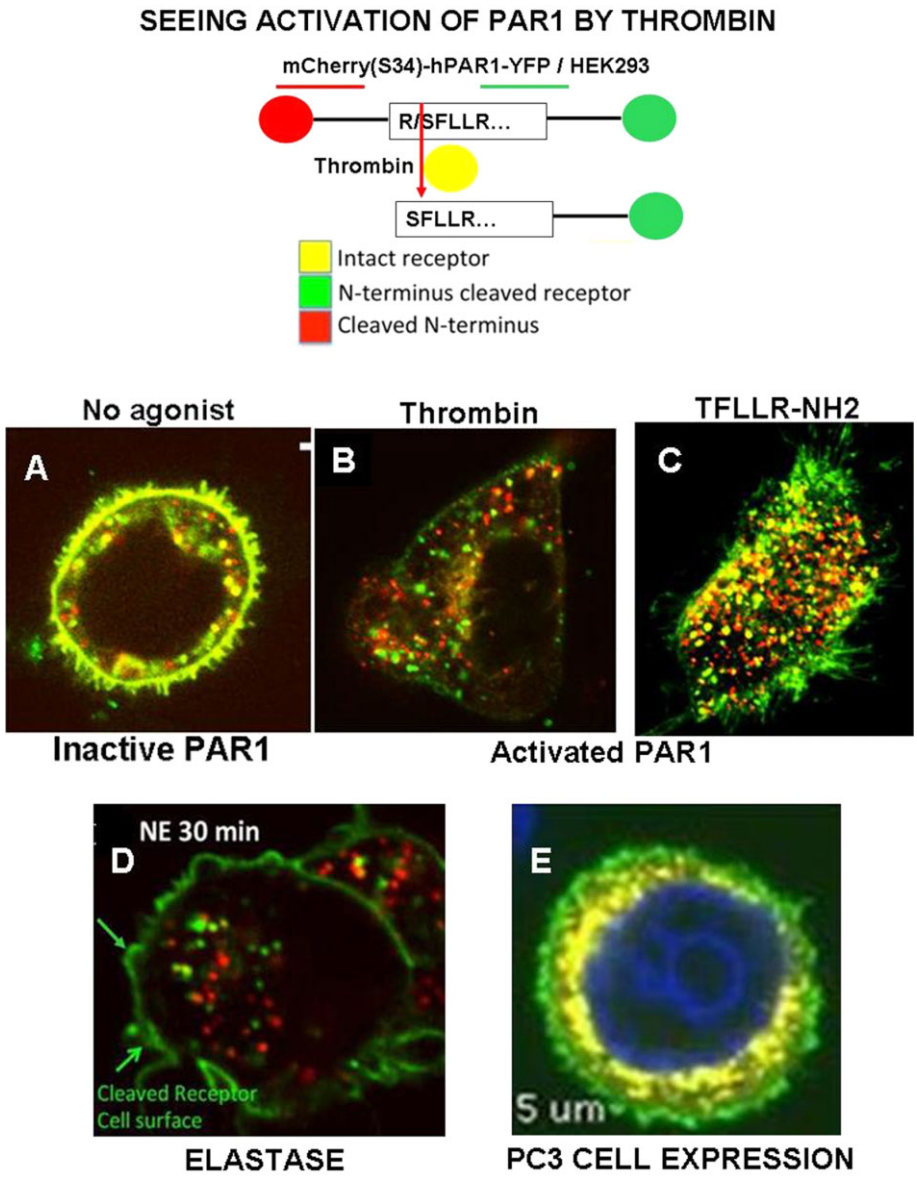

Figure 6

Visualizing activation of dually tagged PAR1. Upper: dually tagged PAR1 for monitoring receptor activation. As shown in the upper cartoon, thrombin cleavage of the dually tagged PAR1 (N-terminal, mCherry; C-terminal YFP: non-activated appearance, 'yellow') releases the mCherry tag so that the remaining C-terminally YFP-tagged activated receptor appears 'green'. Lower: visualizing PAR1 activation in HEK (B–D) and in PC3 cells (E). Panel A shows the non-activated receptor as expressed in HEK that appears largely 'yellow' at the cell surface. Panel B shows that when activated by thrombin, the cleaved-activated YFP-retaining receptor appears as 'green' internalized dots and the released mCherry tag is also internalized (red dots). Panel C shows that when activated non-enzymatically by the PAR1-activating peptide, TFLLR-NH_2, the dual tag is retained on the activated receptor that internalizes as 'yellow' dots [dually tagged PAR1: described by Mihara *et al.* (2013)]. Panel D shows 'biased' activation of dually tagged PAR1 by NE, with retention of the cleaved/activated receptor (green) at the cell surface. Panel E shows that when expressed in prostate cancer-derived PC3 cells, the dually tagged receptor appears 'green' at the cell surface, indicating constitutive cleavage by enzymes secreted into the medium by the PC3 cells. [Adapted from Mihara *et al.* (2013) and Liu *et al.* (2014) with permission.]

cleavage either by its 'traditional' agonist, thrombin, which stimulates both MAPK and calcium signalling or by the 'biased' agonist, neutrophil elastase, which triggers PAR1-mediated MAPK activation, but not calcium signalling (discussed above for PAR2 and shown also for PAR1: Mihara *et al.*, 2013). As illustrated in Figure 6D (lower panel), when activated by neutrophil elastase, the dually tagged PAR1 remains predominantly at the cell surface (green) in the course of activating MAPK signalling over a 30 min time frame. Again, internalization of the released mCherry fragment is

seen along with the internalization of a minority of elastase-activated receptor species (green dots) (Figure 6D, lower panel).

Taken together, our data show that, like other GPCRs, PARs can exhibit biased signalling when activated either by different agonist peptides or by different proteinases that unmask distinct TL sequences (Table 1). Further, the data show that biased signalling appears to originate from distinct membrane environments (e.g. plasma membrane vs. internalized signalling complexes), where the PARs can, in accord with the mobile receptor paradigm outlined in Figure 3, interact with distinct signalling effectors.

PARs as sensors for autocrine–paracrine proteinase-mediated signalling

Visualizing PARs in receptor-expressing tumour-derived prostate cancer cells

When PAR2 was first cloned, its expression was observed in a variety of unexpected locations, including the eye and prostate gland (Nystedt *et al.*, 1994; 1995; Bohm *et al.*, 1996). Knowing that the prostate is a source of the kallikrein-related peptidase (KLK) family of serine proteinases, we proposed that the prostate might potentially host a KLK-PAR autocrine–paracrine system for proteinase-regulated PAR activation. To support that hypothesis, we went on to show that the KLKs can regulate PAR activation and that the KLK family members can be considered as important modulators of cell signalling in the setting of cancer of the prostate and other organs (Oikonomopoulou *et al.*, 2006; 2010; Hollenberg, 2014). With this kind of role in mind, we evaluated the activation status of PAR1 when expressed as a dually tagged receptor in prostate cancer-derived PC3 cells, which originated from a prostate cancer bone metastasis. To our surprise, we saw that the PC3-expressed dually tagged PAR1 is localized as a green receptor at the cell surface (Figure 6E, lower panel) instead of its yellow appearance as an intact receptor (Figure 6A, lower panel). Our preliminary data thus suggest that PC3 cells secrete PAR-regulating proteinases that can, in an autocrine-paracrine way, regulate PC3 PAR signalling. This kind of signalling in the setting of cancer is not yet widely appreciated.

Detecting PAR-regulating proteinases produced by prostate cancer cells

To quantify the presence of PAR-cleaving proteinases secreted by both normal and tumour-derived cells in culture, we have designed a new approach in which PARs 1 and 2 are expressed with an N-terminal fluorogenic (F) tag in place of the mCherry fluorophore shown in Figure 6 (upper panel). When expressed in a background PAR-deficient indicator cell, the N-terminal F-tag can be released into the supernatant by proteinases like thrombin (PAR1) or trypsin (PAR2). Similarly, when exposed to supernatants from either normal or tumour-derived cells (e.g. PC3 or DU145 prostate cancer cells), a release of the N-terminal PAR-attached F-tag into the supernatant serves as an index of the production of PAR-cleaving proteinases. Using this assay, we have been able to detect multiple PAR-cleaving proteinases produced constitutively and released into the growth medium by prostate cancer-derived PC3, DU145 and LNCaP cells (Liu *et al.*, 2014). These preliminary data underline the importance of proteinases and their PAR targets as important localized regulators of tissue function in health and disease, where the PARs can serve as 'sentinels' of tissue inflammation and tumour cell growth and invasion. As summarized in the following sections, this autocrine–paracrine proteinase-mediated process may indeed play a key role in inflammatory processes *in vivo*.

PARs and inflammation

PAR-triggered paw oedema

In view of our hypothesis that locally produced proteinases can act in an autocrine–paracrine way to regulate local tissue function, we proposed that mast cell proteinases would, upon release, trigger a PAR-mediated inflammatory response. To test this hypothesis, instead of using isolated rat mast cell proteinases directly, which proved a challenge to purify to homogeneity, we employed locally administered PAR2-activating peptides to mimic a mast cell proteinase PAR stimulus in a paw oedema inflammation model (Vergnolle *et al.*, 1999). This approach immediately turned our attention to the role of PARs in inflammation as the s.c. administration of PAR-APs caused a robust neurogenic oedematous inflammatory response as shown in Figure 7. This paradigm-shifting experiment, performed over 15 years ago, led to a new view of the potential roles for PARs as an integral component of the innate inflammatory response, involving the rapid induction of pain, neutrophil recruitment, increased perfusion and swelling as reviewed in more detail elsewhere (Ramachandran and Hollenberg, 2008; Vergnolle, 2009). Thus, the PARs joined other receptors like the ones for kinins and complement-derived peptides as receptors involved in driving an inflammatory response. Continuing work was thus focused on evaluating the potential role of PARs in other models of inflammatory disease as outlined in the following sections.

PARs and arthritis

Soon after the discovery that PARs can trigger inflammation in peripheral tissues, it was found that PAR2 is integrally involved in generating joint inflammation in a rodent model of adjuvant-induced arthritis (Ferrell *et al.*, 2003; Kelso *et al.*, 2006). Not only was the adjuvant-induced joint inflammation absent in PAR2 null mice (Ferrell *et al.*, 2003), but treatment of animals with an antibody that shields the PAR2 N-terminus from proteinase cleavage by covering the TL domain (Figure 8) prevents the development of adjuvant-induced joint swelling (Kelso *et al.*, 2006). The data thus indicated not only that PAR2 plays a key role in this model of arthritis but also that PAR2-targeted inhibitors or serine proteinase inhibitors may prove of benefit in arthritic disease as well as in other diseases manifest by tissue inflammation, like colitis.

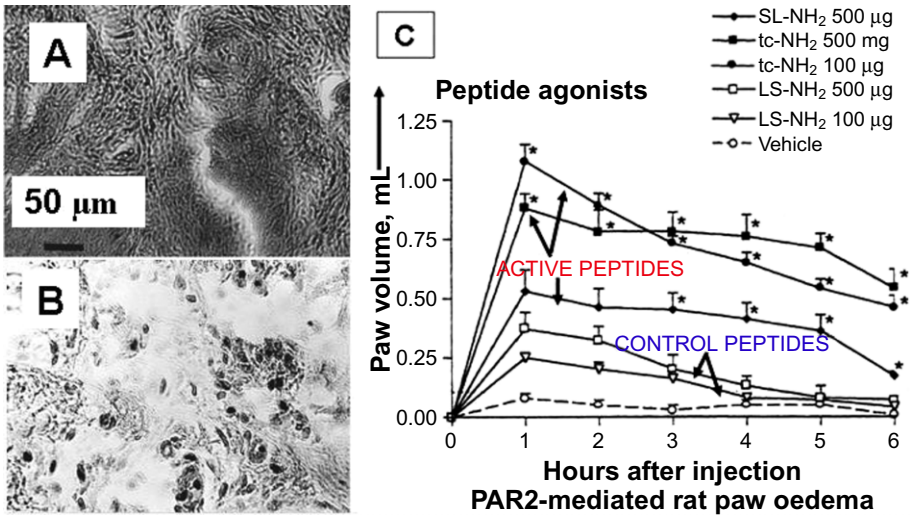

Figure 7

PAR2-induced inflammation: paw oedema. The inflammatory effects of PAR2 activation in the rat paw were evaluated by the intraplantar administration of either the PAR2-activating peptide, SLIGRL-NH_2, or its reverse sequence PAR2-inactive analogue, LRGILS-NH_2. (A and B) Tissue histology: at 6 h after the injection of the control PAR-inactive peptide (LRGILS-NH_2) (A) or the PAR2-activating peptide SLIGRL-NH_2 (B), animals were killed and the injected paws were fixed and stained to evaluate tissue morphology. The scale bar in panel A, for both micrographs = 50 µm. (C) Paw swelling: the time course of swelling after intraplantar administration of the different doses of PAR2-activating peptides shown in the inset, measured using a hydroplethismometer, was observed for up to 6 h at which time animals were killed as described earlier to collect the hindpaw tissue for conducting the histochemical analysis. Swelling was maximal at about 1 h for the active peptides (red font-designated arrows) relative to the PAR2-inactive peptides or peptide-free buffer (blue font-designated arrows). [Adapted from Vergnolle *et al.* (1999) with permission.]

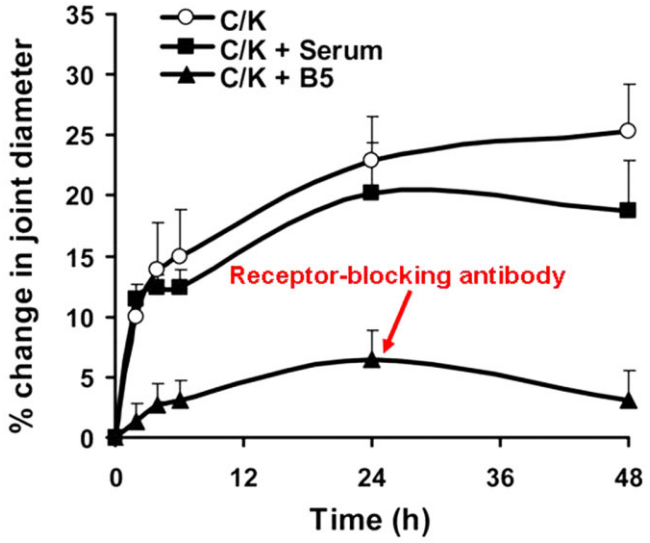

Figure 8

PAR2-blocking antibody attenuates adjuvant-induced arthritis. Adjuvant-induced arthritis was generated by the intra-articular administration of a carrageenan/kaolin (C/K) suspension with or without prior intra-articular administration of a PAR2-targeted antibody (B5) that blocks cleavage activation of PAR2 by proteinases. The C/K-induced increase in joint diameter over a 2 day time frame was markedly diminished by pretreatment with the cleavage-blocking B5 antiserum (C/K + B5), but not by non-immune rabbit serum (CK+serum). [Adapted from Kelso *et al.* (2006) with permission].

PARs as neurogenic mediators of inflammation in the periphery and in the CNS

Using the paw oedema model of inflammation, we learned that there is a substantial role for nerve-released peptide mediators like substance P and calcitonin gene-related peptide in generating the oedema and pain response to PAR activation (Steinhoff *et al.*, 2000). This neurogenic component of the inflammatory response, due to the presence of PARs on sensory nerves, alerted us to the role that neuronal PARs can play. We thus turned our attention to the processes in which PARs might be involved in inflammatory processes in the CNS where PARs are present on both neuronal and non-neuronal cells (Noorbakhsh *et al.*, 2003; Luo *et al.*, 2007).

PAR1 and CNS neuroinflammation

Because we had used locally administered PAR-APs in the rodent paw oedema model, we employed the same strategy for evaluating the local activation of PARs in the CNS. Thus, we monitored the impact of an intracerebral stereotactic striatal administration of a PAR1-activating peptide in mice (Boven *et al.*, 2003). In striatal tissue exposed to a PAR1-activating peptide (but not the sequence-reversed PAR1-inactive peptide), immunohistochemistry revealed evidence of increased inflammatory markers in astrocytes (Figure 9B) and in parenchymal microglial cells (Figure 9D) (Boven *et al.*, 2003). At 7 days after the administration of the TFLLR-NH_2 PAR1-activating peptide (but not the PAR1-inactive reverse sequence peptide), the mice showed neurobehavioural motor symptoms indicative of neurological damage (Boven *et al.*, 2003). In parallel, in brain tissue from individuals with HIV

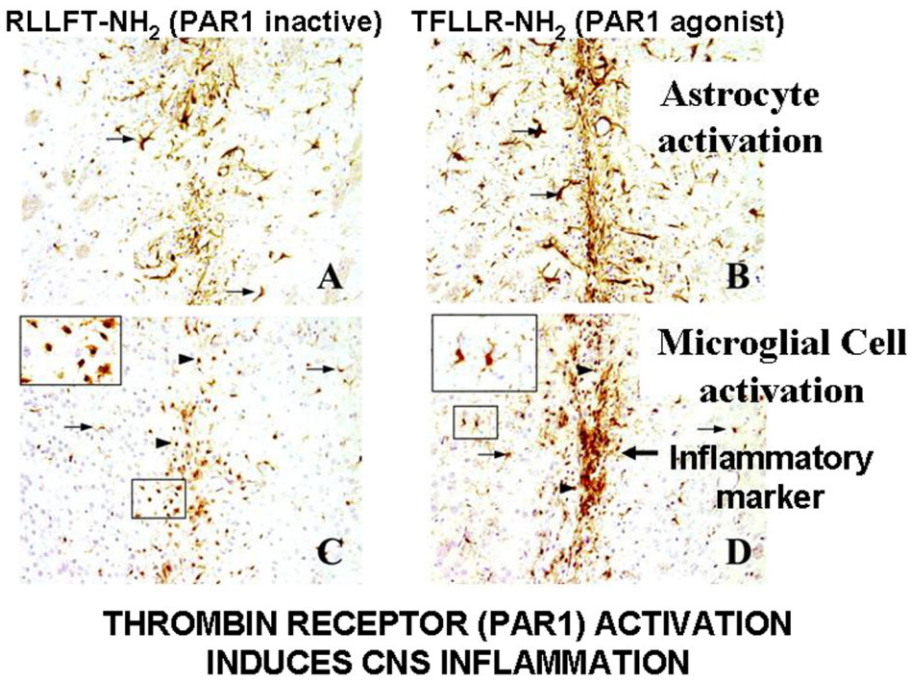

Figure 9

PAR1 activation causes CNS inflammation. A PAR1-selective activating peptide (TFLLR-NH_2: panels B and D) or its reverse sequence PAR1-inactive peptide (RLLFT-NH_2: panels A and C) were administered stereotactically into the striatum of anaesthetized CD-1 male mice. Animals were killed after 7 days and fixed brain sections were prepared for immunohistochemical analysis to detect astrocyte and microglial cell activation (brown staining). Immunohistochemical staining indicative of neuroinflammation was observed in the PAR1-activating peptide-treated tissue (C and D) but was minimal in the reverse peptide-treated tissue (A and B). [Adapted from Boven *et al.* (2003) with permission.]

encephalitis, we found that the abundance of mRNA for both PAR1 and (pro)thrombin were increased in the HIV-derived samples. In contrast, no elevation was observed in samples obtained from individuals with multiple sclerosis (MS). Our data thus implicate a role for PAR1 and its activating proteinases in the setting of CNS inflammation.

PAR2 and MS

As just mentioned, we found that PAR1 mRNA was not up-regulated in tissues from patients with MS. We therefore turned our attention to examine more closely a potential role for PAR2 in the setting of MS. To evaluate a role for PAR2 in this condition, we used an experimental auto-immune encephalitis model in which immunizing mice with a myelin oligodendrocyte glycoprotein (MOG) antigen leads to progressive neurobehavioural dysfunction (Noorbakhsh *et al.*, 2006). Further, we examined PAR2 expression in white matter obtained both from the MS model mice and from humans with MS. For the murine model, we used both wild-type and PAR2 null animals.

In the brain white matter from both humans with MS and from wild-type mice treated with MOG antigen, we found that PAR2 expression was increased on astrocytes and infiltrating macrophages. Furthermore, inflammatory gene expression in the CNS of the MOG-treated PAR2 wild-type animals was greater than in the PAR2 null animals. This increase in inflammatory indices in the wild-type mice correlated with markedly greater microglial activation and greater demyelination compared with the PAR2 null mice. The histopathology findings showed increased CNS inflammation in the wild-type animals correlated with an increased neurobehavioural disability in these animals compared with the PAR2 null littermates (Figure 10) (Noorbakhsh *et al.*, 2006). Our data thus support a role for PAR2 in the process of the inflammatory immune demyelination that is a hallmark of MS. This receptor therefore appears as a potential therapeutic target for the disease.

Which CNS proteinases might trigger PAR-induced neuroinflammation?

With both PARs 1 and 2 implicated in neuroinflammation, a question to be asked is: Which proteinases might regulate the PARs in the nervous system? Enzymes of the coagulation cascade are likely candidates, originating both from the circulation as a result of head trauma or stroke and from the CNS tissues themselves, in which we and others have identified (pro)thrombin mRNA. Another interesting PAR-activating candidate was identified some time ago by reduced stringency PCR cloning as a trypsin-related serine proteinase derived from the rat spinal cord (Scarisbrick *et al.*, 1997). This enzyme, originally termed rat 'myelencephalon specific protease', has turned out to be a member of the KLK family of serine proteinases, namely, KLK6 (Bernett *et al.*, 2002). This enzyme has been found to exacerbate glutamate neurotoxicity via PAR2 activation (Yoon *et al.*, 2013). Of direct relevance to this neurotoxic action of KLK6 and further to our finding

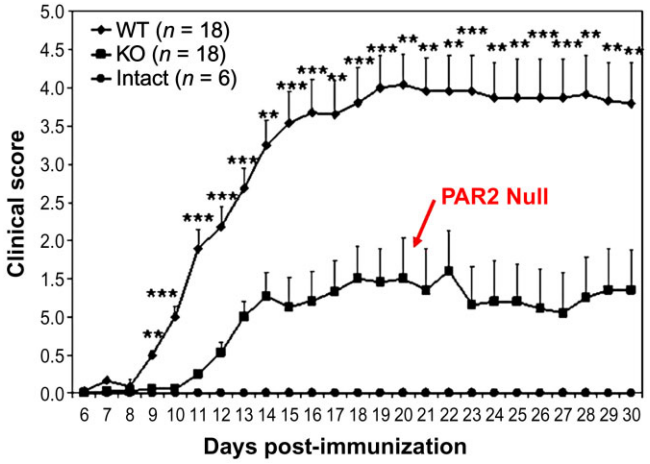

Figure 10

PAR2 null mice with auto-immune encephalitis are protected from neurobehavioural disability. Female wild-type or PAR2 null C57Bl mice were immunized with MOG and neurobehavioural measurements (clinical score) were conducted over a 1 month time frame as disease developed. Non-immunized mice (intact) showed none of the neurobehavioural changes that were seen in the MOG-treated animals. The PAR2 null animals (KO) (red font label and arrow: lower curve) were markedly less affected than their wild-type littermates (WT) (upper curve) indicating a role in the development of this inflammatory model of multiple sclerosis. Adapted from Noorbakhsh *et al.* (2006) with permission.

of a role for PAR2 in MS, we have shown that this enzyme, found in MS lesions both in humans and rodents (Scarisbrick *et al.*, 2002), can activate PAR2 (Oikonomopoulou *et al.*, 2006). Our preliminary immunohistochemistry work with a KLK6-targeted antiserum indicates that KLK6 is found in a variety of cells in the brain. Thus, as we propose for the prostate cancer cells described earlier, we suggest that in the CNS a 'paracrine–autocrine' loop might regulate tissue function involving the secretion activation of KLK6 or other proteinases, which in turn can activate PAR2.

Summing up

About the PARs

To summarize, it was the parallel I perceived between the anabolic/mitogenic actions of thrombin and those of EGF that led to my shift from work with the EGF receptor (Hollenberg and Cuatrecasas, 1973; 1975; Zheng *et al.*, 1998) to focus on the thrombin receptor in the early 1990s. Using our vascular and gastric bioassays that responded both to EGF and thrombin receptor activation, we were thus poised to become involved in work on the molecular pharmacology of the PAR family of GPCRs (Coughlin, 2005; Adams *et al.*, 2011).

What may not be visible to the outside observer is the essential impact on this research direction provided by the training environment I experienced in the Oxford Department led by Paton. Thus, the vision Paton promoted, 'that the chemistry embedded in the discipline of pharmacology can progress from the molecular level to the whole man', led not only to a study of the detailed molecular pharmacology of the PARs but also to an evaluation of the potential roles of the PARs in a number of settings of inflammatory pathophysiology. That work aims to identify therapeutic targets to mitigate disease. Indeed, despite the challenges of dealing with a proteolytic TL mechanism of receptor activation, PAR-targeted antagonists with therapeutic potential have been developed, although their clinical utility has yet to be fully realized (Ramachandran *et al.*, 2012). Further work on PAR antagonist development is currently ongoing with the projection that such compounds will find a place in dealing with conditions like arthritis, colitis, atopic dermatitis, CNS neurodegeneration and tumour invasion metastasis. This possible therapeutic outcome for inflammatory disease would be a direct consequence of the South Parks Road Oxford Department environment.

The Paton-led department and its impact on other departments: a continuing legacy

Clearly, I owe an immense debt to the 'house that Paton built' in terms of the training environment that I found there from 1964 to 1968. But, there is also much more in terms of the impact on others whom I met at that time. In the corridors there were individuals who, including myself, went on to head up (count them) five Departments of Pharmacology (institutions in alphabetical order): Innsbruck Medical University (Hans Winkler), Oxford University (David Smith), University College London (Humphrey Rang), University of Alberta (David Cook) and the University of Calgary (myself, shared in part with Sheldon Roth, who also trained with Paton in the Oxford Department from 1971 to 1973). I would throw out the challenge for *any* department to match that legacy to be achieved over such a short time frame. In all of those departments, the 'Paton imprint' continues to foster the discipline of pharmacology and therapeutics.

Also, looking forward from the 1960s, one can point to the substantial impact that individuals trained in the Oxford Department have had to date on the development of therapeutic agents. One can anticipate more progress in that area to be generated in the near future. To document those contributions is unfortunately beyond the scope of this synopsis. All said and done, however, one can see how the foundation that Paton provided for us in the Department on South Parks Road, Oxford, has flourished, no doubt well beyond his expectations, to spearhead developments in pharmacology and therapeutics well into the future.

Acknowledgements

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of the PARs and their activating proteinases. Whatever progress has been made is a tribute to the cooperative atmosphere in which my colleagues and I are able to work. This collaborative approach reflects the one I inherited from my time in the Oxford Department. I am also indebted to the editors and reviewers of this manuscript who have made suggestions that have substantially improved the quality of this overview.

Conflict of interest

The author asserts that there is no conflict of interest related to the information included in this article.

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