BJP British Journal of Pharmacology

DOI:10.1111/j.1476-5381.2012.02234.x
www.brjpharmacol.org

REVIEW

Roles of proteolysis in regulation of GPCR function

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Keywords
peptidase; neuropeptide; GPCR; trafficking; signalling; proteolysis; endosome; cell-surface; ubiquitin; β-arrestins

Received
11 July 2012
Revised
3 September 2012
Accepted
24 September 2012

The enzymatic activity of peptidases must be tightly regulated to prevent uncontrolled hydrolysis of peptide bonds, which could have devastating effects on biological systems. Peptidases are often generated as inactive propeptidases, secreted with endogenous inhibitors, or they are compartmentalized. Propeptidases become active after proteolytic removal of N-terminal activation peptides by other peptidases. Some peptidases only become active towards substrates only at certain pHs, thus confining activity to specific compartments or conditions. This review discusses the different roles proteolysis plays in regulating GPCRs. At the cell-surface, certain GPCRs are regulated by the hydrolytic inactivation of bioactive peptides by membrane-anchored peptidases, which prevent signalling. Conversely, cell-surface peptidases can also generate bioactive peptides, which directly activate GPCRs. Alternatively, cell-surface peptidases activated by GPCRs, can generate bioactive peptides to cause transactivation of receptor tyrosine kinases, thereby promoting signalling. Certain peptidases can signal directly to cells, by cleaving GPCR to initiate intracellular signalling cascades. Intracellular peptidases also regulate GPCRs; lysosomal peptidases destroy GPCRs in lysosomes to permanently terminate signalling and mediate down-regulation; endosomal peptidases cleave internalized peptide agonists to regulate GPCR recycling, resensitization and signalling; and soluble intracellular peptidases also participate in GPCR function by regulating the ubiquitination state of GPCRs, thereby altering GPCR signalling and fate. Although the use of peptidase inhibitors has already brought success in the treatment of diseases such as hypertension, the discovery of new regulatory mechanisms involving proteolysis that control GPCRs may provide additional targets to modulate dysregulated GPCR signalling in disease.

Abbreviations

ADAM, a disintigrin and metallopeptidase; CGRP, calcitonin gene-related peptide; CLR, calcitonin receptor-like receptor; CRF_1 receptor, corticotropin-releasing factor 1 receptor; CXCR, C-X-C chemokine receptor; ECE-1, endothelin-converting enzyme-1; EGF, epidermal growth factor; HB-EGF, membrane-anchored heparin-binding EGF-like growth factor; NEP, neprilysin; NK receptor, neurokinin receptor; NTS_1 receptor, neurotensin 1 receptor; PAR, proteinase-activated receptor; PP2A, protein phosphatase 2A; RAMP, receptor activity-modifying protein; sst receptor, somatostatin receptor; SP, substance P; USP, ubiquitin-specific peptidase; Unc1, urocortin 1

Nomenclature

For receptors, the British Journal of Pharmacology's *Guide to Receptors and Channels* was used (Alexander *et al.*, 2011). For peptidases, the MEROPS database was used (Rawlings *et al.*, 2012).

The traditional notion of proteolysis

Proteolysis is the hydrolytic breakdown of a peptide bond, C(O)-NH found between amino acids in peptides, polypeptides and protein structures. Peptide bonds can spontaneously break in the presence of water, but do so, at an extremely slow rate. Therefore, in biological systems, enzymes are required to facilitate the breakage of these bonds. These enzymes or more specifically, peptidases (also known as proteases or proteinases) are themselves made of amino acids and are currently classified into six groups according to the critical residue in their catalytic site: (i) serine peptidases; (ii) cysteine peptidases; (iii) aspartic peptidases; (iv) threonine peptidases; (v) metallopeptidases; and (vi) glutamic peptidases (Rawlings *et al.*, 2012). Traditionally, peptidases are mainly thought of as enzymes of digestion,

breaking down food in the stomach and in the intestine. However, it is now clear that peptidases can contribute to the regulation of cell function by controlling levels of bioactive peptides and by cleaving cell-surface receptors and ion channels to regulate signalling pathways.

Cell-surface peptidases regulate the availability of GPCR ligands

The role of cell-surface peptides in the regulation of GPCR signalling is well documented. These peptidases regulate levels of circulating bioactive peptides, which function to initiate GPCR-mediated signalling. ACE compound peptidase (EC 3.4.15.1) is a zinc-dependent metallopeptidase that converts the inactive angiotensin I to angiotensin II by releasing the C-terminal dipeptide, His^{9}-Leu^{10} (Figure 1A). Angiotensin II is a vasoconstrictor and exerts its effect through two types of angiotensin II receptors, AT_1 and AT_2. The AT_1 receptor mediates most of the physiological and pathophysiological actions of angiotensin II and is the predominant receptor subtype expressed in the cardiovascular system. Interaction of the AT_1 receptor with angiotensin II activates $G_{q/11}$, G_i, G_{12} and G_{13} proteins, leading to the mobilization of intracellular calcium, generation of reactive oxygen species and activation of numerous PKs and mitogenic signalling pathways [reviewed in Mogi *et al.* (2009)]. AT_2 receptors couple to G proteins to activate PLC, promoting the mobilization of intracellular calcium and activation of PKC [reviewed in Porrello *et al.* (2009)]. Angiotensin II causes a plethora of effects, including tissue remodelling, leukocyte infiltration, inflammation, atherosclerosis, endothelial dysfunction, myocardial infarction, stroke, and heart and renal failure [reviewed in Cheng *et al.* (2005)]. Similarly, endothelin-converting enzyme-1 (ECE-1, EC 3.4.24.71) is responsible for the production of the vasoconstrictor endothelin-1 from big endothelin. Endothelin-1 can activate both endothelin A (ET_A) and ET_B receptors to elicit a broad range of signalling responses [reviewed in Khimji and Rockey (2010)]. Activation of ET_A receptors by endothelin-1 promotes mobilization of intracellular calcium, indicating that ET_A receptors are coupled to $G_{q/11}$ proteins. ET_A receptors primarily mediate the vasoactive and proliferative effects of endothelin-1. ET_B receptors have been proposed to act as endothelin-1 scavengers and thus, reduce circulating endothelin-1 levels. Thus, proteolysis acts to promote activation of GPCRs. In contrast, proteolysis can also act to prevent activation of GPCRs. For example, neprilysin (NEP, neutral endopeptidase 24.11, EC 3.4.24.11) cleaves and inactivates both bradykinin (Gly^{4}↓Phe^{5} and Pro^{7}↓Phe^{8}) and substance P (SP, Gln^{6}↓Phe↓Phe-Gly↓Leu^{10}) (Matsas *et al.*, 1984), thus preventing activation of their respective GPCRs, the bradykinin 2 receptor (B_2 receptor) and the neurokinin 1 receptor (NK_1 receptor) (Figure 1A). Peptidases can therefore play a major role in the production of vasoactive peptides and, therefore, regulate vascular functions and dysregulation can lead to vascular diseases. A great deal of time and effort has been spent on the development of peptidase inhibitors for the treatment of hypertension. It is over 30 years since the first ACE inhibitor, captopril, was designed (Ondetti *et al.*, 1977). Now, however, other ACE inhibitors with improved pharmacokinetics and pharmacodynamics have since been developed and include enalaprilat (MK-421) (Gross *et al.*, 1981) and imidaprilat (Ikeo *et al.*, 1992). ACE inhibitors are effective treatments for hypertension and congestive heart failure (CONSENSUS, 1987; SOLVD, 1991) and are also beneficial for patients with atherosclerosis (Yusuf *et al.*, 2000). With the success of ACE inhibitors for the treatment of hypertension, it was thought that the development of compounds able to inhibit multiple peptidases, thereby potentiating the effects of dilator peptides such as bradykinin, while reducing the availability of constrictors such as angiotensin II, may offer an even better way of treating diseases such as hypertension. Indeed, dual or triple vasoactive peptidase inhibitors have been developed. These compounds inhibit the proteolytic activities of ACE, ECE-1 and NEP in various combinations. The first described dual inhibitors were alatriopril and glycoprilat. Both compounds inhibit the activities of both ACE and NEP (Gros *et al.*, 1991). Alatriopril was shown to be more effective than captopril alone in reducing cardiac hypertrophy in rats with myocardial infarction (Bralet *et al.*, 1994). Later, omapatrilat (BMS-186716) was developed and was shown to

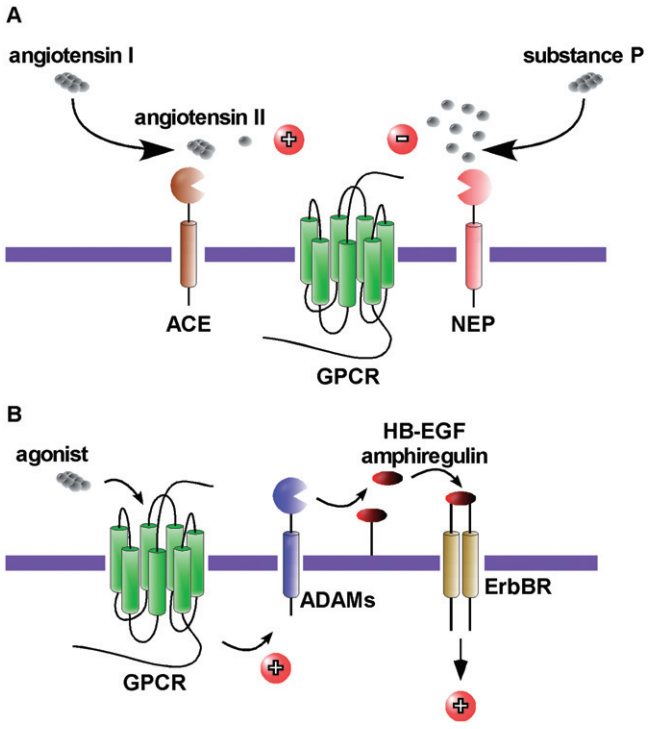

Figure 1

Cell-surface peptidases regulate GPCR-mediated signalling. (A) ACE compound peptidase cleaves angiotensin I to generate angiotensin II to promote activation of angiotensin II receptors. Conversely, NEP hydrolyses SP to prevent activation of SP of neurokinin 1 receptors. (B) Activation of certain GPCRs promotes the ADAMs that generate EGF-like ligands (e.g. heparin-binding EGF-like factor, amphiregulin). In turn, these ligands transactivate ErbB receptors to activate intracellular signalling pathways.

Table 1

Future and current uses for peptidase inhibitors in the treatment of disease

Peptidase	Drug perspectives	Targeted GPCRs	References/reviews
Cell-surface			
ACE, ECE-1, NEP[a]	Hypertension	AT_1, AT_2, ET_A, ET_B, B_2	Dive *et al.* (2009)
NEP, aminopeptidase N	Pain	Opioid receptors	Thanawala *et al.* (2008)
ADAMs	Cancer progression	GPCRs causing ErbB receptor transactivation	Paolillo and Schinelli (2008)
Extracellular			
Thrombin, Coagulation Factor Xa	Anticoagulants, thrombosis	PAR_1, PAR_3, PAR_4	Showkathali and Natarajan (2012)
Trypsin, Tryptase	Inflammatory bowel diseases	PAR_2	Rothmeier and Ruf (2012; Yoshida and Yoshikawa (2008)
Lysosomal			
Cathepsins B, C, D, E, G and L, Carboxypeptidase A	No known functional consequences of preventing GPCR degradation	–[b]	N/A
Endosomal			
ECE-1	Modulation of GPCR trafficking and signalling	NK_1, CLR•RAMP1, sst_2, NT_1, CRF_1	Murphy *et al.* (2009; 2011)
USPs	Modulation of GPCR trafficking and signalling	GPCR function affected by de-ubiquitination of β-arrestins	Murphy *et al.* (2009; Shenoy *et al.* (2009)

[a]Dual and triple ACE, ECE-1 and NEP inhibitors are in development.
[b]Too numerous to list in table. N/A, not applicable.

be more effective in reducing blood pressure in humans than either placebo or ACE inhibitors (Neal *et al.*, 2002; Regamey *et al.*, 2002). However, omapatrilat failed in phase III clinical trials and was discontinued due to an increased incidence of angioedema as an unwanted side effect. CGS 35601, a triple vasopeptidase inhibitor, prevents the activities of ACE, NEP and ECE-1 (Trapani *et al.*, 2004) and significantly reduced both systolic and diastolic blood pressure in a number of preclinical rodent models of hypertension (Daull *et al.*, 2005; 2006a). Further, a preclinical safety profile assessment of CGS 35601 showed it to have no effect on either hepatic or liver toxicities (Daull *et al.*, 2006b). Although these triple inhibitors may represent the future of peptidase inhibitors for the treatment of disease (Table 1), no clinical trials using triple peptidase inhibitors have yet been conducted. So, although these dual and triple peptidase inhibitor compounds are promising in humans and animal models of hypertension, none have yet been approved for the treatment of human disease. Thus, ACE inhibitors remain the compounds of choice for the treatment of hypertension, often in combination with angiotensin or β-adrenoceptor antagonists or diuretics. NEP also plays a major role in the catabolism of endogenous opioid peptides such as the enkephalins and dynorphins. Thus, together with aminopeptidase N (EC 3.4.11.2), NEP represents a major target for the development of drugs for the treatment of acute and chronic pain [reviewed in Thanawala *et al.* (2008)].

GPCRs promote proteolysis to transactivate epidermal growth factor receptors

Certain GPCRs can activate intracellular signalling pathways via transactivation of cell-surface receptor tyrosine kinases such as PDGF (Linseman *et al.*, 1995), insulin-like growth factor-1 receptor (Rao *et al.*, 1995), ErbB receptor (Daub *et al.*, 1996) and Trk neurotrophin receptors (Lee and Chao, 2001). Interestingly, only transactivation of ErbB receptors requires the activity of peptidases (Figure 1B). The GPCR agonists, endothelin-1, lysophosphatic acid and thrombin, were shown to induce phosphorylation of the ErbB receptor in Rat-1 cells (Daub *et al.*, 1996). Specific inhibition of ErbB receptors using the selective tyrphostin AG1478 or expression of a dominant-negative ErbB receptor mutant that is unable to signal, suppressed the GPCR-induced activation of ERK1/2 (Daub *et al.*, 1996). Later studies revealed that transactivation of ErbB receptors, involves the processing of membrane-anchored heparin-binding EGF-like growth factor by a metalloproteinase activity that is rapidly induced following GPCR activation. Inhibition of the peptidase activity using the metalloproteinase inhibitor batimastat prevented ErbB receptor transactivation and downstream signals (Prenzel *et al.*, 1999). Additional work, indicated that this GPCR-mediated transactivation not only lead to the initiation of signalling cascades, but also ErbB receptor dimerization, tyrosine autophosphor-

ylation and internalization (Maudsley *et al.*, 2000). The identity of the metallopeptidase responsible for the processing of the membrane-anchored ligand remained elusive until a study conducted in cardiomyocytes showed that the metallopeptidase inhibitor, KB-R7785 and a dominant-negative of a disintigrin and metallopeptidase (ADAM) 12 peptidase attenuated GPCR-induced signalling (Asakura *et al.*, 2002). The same study also showed that KB-R7785 inhibited the shedding of heparin-binding EGF-like growth factor and attenuated thoracic aortic constriction-induced thickening of the heart muscle in intact mice. A subsequent study suggested that ADAM10 peptidase (EC 3.4.24.81) is also implicated in bombesin-mediated ErbB receptor transactivation (Yan *et al.*, 2002). As more than one peptidase is involved in ErbB receptor transactivation, it came as no surprise that additional ligands were also involved. In squamous cell carcinoma cells, the GPCR agonists, lysophosphatic acid and carbachol, which activate lysophospholipid and muscarinic receptors, respectively, specifically activate metallopeptidase-dependent release of amphiregulin by another ADAM peptidase, ADAM17 peptidase (TNF-α-converting enzyme, TACE; EC 3.4.24.86) to regulate proliferation and motility (Gschwind *et al.*, 2003). However, in TccSup cancer cells, lysophosphatic acid-induced transactivation is mediated by ADAM15 peptidase and promotes cell survival (Schafer *et al.*, 2004).

Following on from the fact that GPCRs can promote the activity of many different metallopeptidases to cause ErbB receptor transactivation, evidence exists for the involvement of numerous GPCR-induced pathways in the generation of EGF-like ligands [reviewed in Ohtsu *et al.* (2006)]. The β_2-adrenoceptor agonist, isoprenaline causes ErbB receptor transactivation via a mechanism that requires Gβγ subunits and c-Src activity (Pierce *et al.*, 2001). Similarly, glucagon-like peptide-1-induced transactivation also requires c-Src activity (Buteau *et al.*, 2003). The involvement of G proteins has also been observed in other studies. Lysophosphatic acid-mediated transactivation by ADAM17 was partially blocked by pertussis toxin in squamous cell carcinoma-9 cells (Gschwind *et al.*, 2003) and in MDA-MB-231 cells, lysophosphatic acid- and sphingosine-1 phosphate-dependent ErbB receptor transactivation involving ADAM15, was also prevented by pertussis toxin (Hart *et al.*, 2005). A requirement for the activity of phospholipase C has also been observed for angiotensin II-induced, ADAM17-dependent ErbB receptor transactivation by the AT_1 receptor (Mifune *et al.*, 2005). The elevation of intracellular calcium levels by bradykinin (Zwick *et al.*, 1999) and angiotensin II (Eguchi *et al.*, 2003) has also been implicated in the transactivation of ErbB receptors. It is assumed that many of these GPCR-induced pathways lead to the phosphorylation of the metallopeptidases, enhancing the proteolytic activity of the peptidase. Although it is well established that numerous PKs can phosphorylate metallopeptidases to promote proteolytic activity [reviewed in Huovila *et al.* (2005)], none of the present studies have presented evidence of a direct GPCR-mediated effect on metallopeptidases.

The generation of reactive oxygen species by GPCRs has also been proposed to play an important role in promoting metallopeptidase activity and includes ErbB receptor transactivation mediated by $5\text{-}HT_{2B}$ receptors and α_{1D}-adrenoceptors (Pietri *et al.*, 2005), AT_1 receptor (Eguchi and Inagami, 2000) and purine P2Y receptors (Myers *et al.*, 2009). The reactive oxygen species are thought to induce oxidation of a cysteine residue that lies within an inhibitory motif, thereby activating the peptidase (Zhang *et al.*, 2001). A more recent development in elucidating the mechanism by which metallopeptidases become active following GPCR activation to promote ErbB receptor activation is the involvement of integrins (Gooz *et al.*, 2012). 5-HT induces kidney mesangial cell proliferation through ADAM17 activation and ErbB receptor transactivation. In unstimulated cells, ADAM17 binds to $\alpha_5\beta_1$ integrin, an interaction that prevents the proteolytic activity of ADAM17. However, following application of 5-HT, this interaction is disrupted, presumably promoting peptidase activity (Gooz *et al.*, 2012).

A further consequence of the processing of EGF-like ligands from cell-surface precursors, is the generation of C-terminal fragments. It is now established that these C-terminal fragments also have a biological role [reviewed in Tanida *et al.* (2010)]. IL-8 induces cell proliferation and migration of the colon cancer cell lines, HT-29 and Caco2 by an ADAM-dependent intranuclear translocation pathway of HB-EGF-C-terminal fragment (Itoh *et al.*, 2005). Thus, understanding how GPCRs activate cell-surface peptidases to generate the multiple ligands responsible for receptor transactivation is essential to develop new pharmacological interventions. The importance of metallopeptidases in generating EGF-like ligands that regulate cell proliferation and differentiation, has lead these peptidases to be considered as useful molecular targets in the treatment of cancer. Specific peptidase inhibitors may also be used to modulate GPCR-induced signalling and this may be an effective treatment for GPCR-driven diseases.

Peptidases can act as positive or negative regulators of GPCR signalling

It was long recognized that peptidases such as thrombin (EC 3.4.21.5) and trypsin could signal directly to cells (Burger, 1970; Sefton and Rubin, 1970; Chen and Buchanan, 1975; Carney and Cunningham, 1977, 1978). However, the mechanism through which these peptidases could activate intracellular signalling cascades remained unknown until the cloning of the thrombin receptor, now called proteinase-activated receptor 1 (PAR_1) (Vu *et al.*, 1991). PAR_1 is a GPCR and thrombin activates PAR_1 by direct cleavage of the receptor in a two-step process. Firstly, thrombin binds to PAR_1 either side of the proteolytic cleavage site. One of these sites ($D^{51}KYEPF^{56}$) is similar to that of hirudin, an anticoagulant found in the saliva of leech (Vu *et al.*, 1991). This hirudin-like binding domain increases the affinity of thrombin for PAR_1. Following binding, thrombin cleaves between Arg^{41} and Ser^{42} to expose the new N-terminus starting with $S^{42}FLLRN^{47}$ (Vu *et al.*, 1991). This tethered ligand domain then interacts with residues on the second extracellular loop of the receptor and presumably induces a conformational change, which activates the receptor. PAR_1 activation by thrombin is the most potent known trigger for platelet aggregation (Sambrano *et al.*, 2001) and as such, thrombin signalling and PAR_1 are key targets for the prevention of thrombosis.

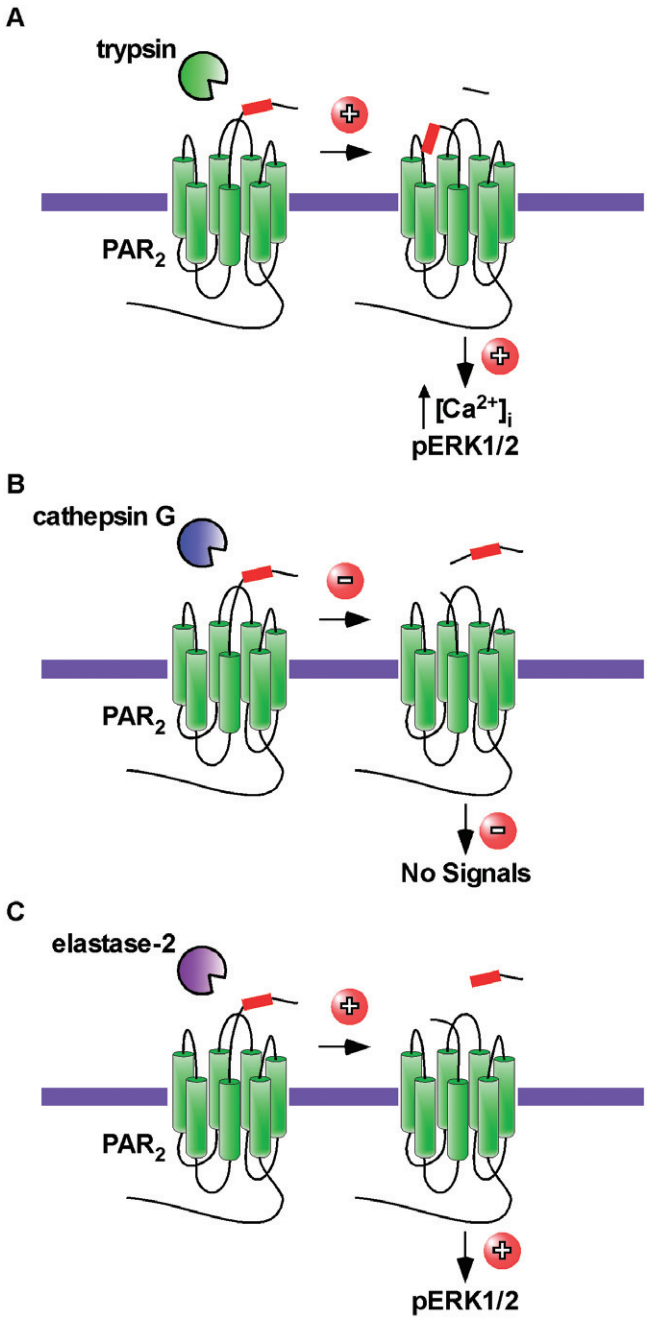

Figure 2

Peptidases act as biased agonists at GPCRs. (A) Trypsin cleaves PAR_2 to create a new N-terminus that activates PAR_2 mobilizing intracellular calcium and promoting phosphorylation (p) of ERK1/2. (B) Cathepsin G cleaves PAR_2, but does not elicit any known signalling and prevents activation by other peptidases such as trypsin. (C) Other peptidases such as elastase-2, cleave PAR_2 at a site distinct from trypsin. The action of this peptidase does not mobilize intracellular calcium, but does activate ERK1/2.

PAR_2 is the second member of this receptor family and is activated by trypsin (Figure 2A) (Nystedt *et al.*, 1994; 1995a,b; Bohm *et al.*, 1996). Cleavage of PAR_2 occurs between Arg^{36} and Ser^{37} to reveal the tethered ligand and new amino terminus of $S^{37}LIGKV^{42}$. The use of PAR_2-specific agonistic and antagonistic peptides and studies in PAR_2-deficient mice, have helped to identify critical roles for PAR_2 in inflammation, development, angiogenesis and immune responses [reviewed in Rothmeier and Ruf (2012)]. Subsequently, two other members of this subfamily of GPCRs have been cloned, PAR_3 (Ishihara *et al.*, 1997; Scase *et al.*, 1997) and PAR_4 (Kahn *et al.*, 1998; Xu *et al.*, 1998), both of which are activated by thrombin. PAR_4 can also be activated by trypsin (Xu *et al.*, 1998). Due to their sensitivity to thrombin, both PAR_3 and PAR_4 are important in platelet function (Ishihara *et al.*, 1998; Kahn *et al.*, 1998).

PARs are promiscuous receptors and may be activated by multiple peptidases. For example, PAR_1 can also be activated by coagulation factor Xa (EC 3.4.21.6) (Camerer *et al.*, 2000), activated protein C (EC 3.4.21.69) (Riewald *et al.*, 2002), matrix metallopeptidase-1 (EC 3.4.24.7) (Boire *et al.*, 2005) and plasmin (EC 3.4.21.7) (Mannaioni *et al.*, 2008). Similarly, PAR_2 is also cleaved by multiple peptidases, including mast cell tryptases (Molino *et al.*, 1997), coagulation factor VIIa (EC 3.4.21.21) in complex with tissue factor and coagulation factor Xa (Camerer *et al.*, 2000), matriptase-1 (membrane-type serine peptidase 1) (Takeuchi *et al.*, 2000) and trypsin IV (Cottrell *et al.*, 2004). PAR_4 is cleaved and activated by trypsin (Xu *et al.*, 1998), cathepsin G (Sambrano *et al.*, 2000), trypsin IV (Cottrell *et al.*, 2004) and plasmin (Quinton *et al.*, 2004). Peptidases that cleave PARs to disarm the receptor and prevent subsequent activation by removing the tethered ligand have also been identified. For example, cathepsin G (EC 3.4.21.20) (Dulon *et al.*, 2003), pseudolysin (EC 3.4.24.26) (Dulon *et al.*, 2005) and myeloblastin (neutrophil leukocyte proteinase 3, EC 3.4.21.76) (Ramachandran *et al.*, 2011) cleave PAR_2 downstream of the tryptic cleavage site inactivating the receptor (Figure 2B). Cathepsin G and elastase-2 (neutrophil elastase, EC 3.4.21.37) abolish signalling by thrombin in PAR_3-transfected cells, and thus disarm PAR_3 (Cumashi *et al.*, 2001).

The concept of biased agonism at GPCRs is not a new one [reviewed in Urban *et al.* (2007)]. Given that PARs are activated by many different peptidases, it is not surprising that the generation of different N-termini can result in peptidase-specific signalling. It is these unique N-termini that act as intramolecular ligands causing specific GPCR conformations to elicit biased signalling responses. The earliest indications that PARs could be selectively activated came from observations comparing activation by synthetic ligands to peptidases. For PAR_1, mutations in the extracellular regions resulted in differential signalling between synthetic peptide agonists and thrombin (Blackhart *et al.*, 2000). For example, a PAR_1 deletion mutant lacking amino acids 68–93 of the N-terminus, failed to mobilize intracellular calcium in response to the synthetic peptide, but retained the ability to respond to thrombin (Blackhart *et al.*, 2000). A subsequent study using human endothelial cells showed that thrombin favours $G_{12/13}$ signalling and induction of endothelial barrier permeability rather than intracellular calcium mobilization, whereas synthetic peptides preferentially caused intracellular calcium mobilization by triggering G_q signalling (McLaughlin *et al.*, 2005). Studies have also shown that activation of PAR_1 by different peptidases can have different functional consequences for endothelial barrier permeability (Feistritzer and

Riewald, 2005; Finigan *et al.*, 2005). Whereas thrombin-dependent cleavage of PAR_1 disrupts endothelial barrier integrity, activated protein C-dependent activation of PAR_1 enhances endothelial barrier function (Feistritzer and Riewald, 2005; Finigan *et al.*, 2005). Challenging human lung epithelial cells with elastase-2 leads to PAR_1-mediated apoptosis, similar to that observed with thrombin and a synthetic-activating peptide, but modified kinetics (Suzuki *et al.*, 2005; 2009). Matrix metallopeptidase-1 cleaves PAR_1 at a distinct site to thrombin and activates Rho-GTP and mitogenic pathways in a biased mechanism, to promote cell shape change and motility (Trivedi *et al.*, 2009).

Agonist-biased signalling has also been observed for PAR_2. Certain synthetic peptide agonists fail to cause PAR_2-mediated calcium signalling, while still activating ERK1/2, whereas SLIGRL-NH_2, which mimics the naturally unmasked tethered ligand, triggers both calcium and MAP kinase signalling (Ramachandran *et al.*, 2009). Further evidence came from the observation that a novel peptidomimetic PAR_2 antagonist, K-14585 activated ERK1/2 signalling, but failed to elicit and calcium responses (Goh *et al.*, 2009). It was initially thought that elastase-2 also disarmed PAR_2 (Dulon *et al.*, 2003). However, recent evidence suggests that elastase-2 is in fact a biased agonist of PAR_2 (Ramachandran *et al.*, 2011) (Figure 2C). Although elastase-2 does not cause internalization of PAR_2 or mobilization of intracellular calcium, it does initiate activation of the ERK1/2 pathway (Ramachandran *et al.*, 2011). The exact mechanism by which peptidases can act as biased agonists has not yet been fully established. However, in the case of PAR_2 it is known that trypsin and elastase-2 cleave PAR_2 at different positions within the N-terminus. Therefore, it is highly probable that the different cleavages result in different conformations of PAR_2, which leads to biased activation of signalling pathways. With regard to PAR_1, although the peptidases may cleave at the same sites, the fact that thrombin actually binds to PAR_1 may lead to slight conformation changes, which also lead to biased activation of signalling cascades. However, until the crystal structures of the activated GPCRs are solved, this is pure conjecture. It is not yet known if biased agonism of PAR_3 and PAR_4 occurs. However, with numerous peptidases able to cleave members of this subfamily of GPCRs and perhaps each with different functional consequences, there is still much to discover about how peptidases signal through GPCRs.

Lysosomal peptidases degrade GPCRs to irrevocably terminate GPCR signalling

Once activation of a GPCR has occurred, many GPCRs are removed from the cell-surface to prevent uncontrolled signalling. The receptors are then either recycled back to the cell-surface mediating resensitization to allow cells to respond to the same stimulus again, or they are trafficked to intracellular compartments, such as lysosomes for degradation, which results in permanent signal arrest. The peptidases present in lysosomes mainly belong to the aspartic, cysteine and serine peptidase classes, with few metallo- and threonine peptidases residing in lysosomes. They are also mainly endopeptidases, cleaving within polypeptide chains and not at the end or beginning of chains. Examples of peptidases localized to lysosomes include the serine peptidases, serine carboxypeptidase A (EC 3.4.16.5) and cathepsin G, aspartic peptidases, cathepsin D (EC 3.4.23.5) and E (EC 3.4.23.24) and cysteine peptidases, cathepsin B (EC 3.4.22.1) and L (EC 3.4.22.15). One mechanism by which many GPCRs are targeted for degradation is by the post-translational modification of ubiquitination, although this is not an absolute requirement for all GPCRs. Once activated, ubiquitin moieties are added to intracellular facing lysine residues by a process requiring three enzymes, an ubiquitin-activating enzyme (E1), an ubiquitin-conjugating enzyme (E2) and an ubiquitin ligase (E3). Before the GPCR is degraded by peptidases present in the lysosomes, the ubiquitin molecules are removed by metallo- or cysteine peptidases, referred to as de-ubiquitinating enzymes or ubiquitin-specific peptidases (USPs). In fact it is an absolute requirement that the ubiquitin molecules are removed before the GPCR can enter the lysosome. The first identified GPCR to be regulated by ubiquitination was the yeast GPCR, Ste2p. Ubiquitination of Ste2p is a requirement for internalization (Hicke and Riezman, 1996). Prolonged exposure of β_2-adrenoceptors to agonists promotes sorting of receptors to lysosomes and degradation by lysosomal peptidases (Moore *et al.*, 1999). A combination of the aspartic peptidase inhibitor, pepstatin A and the serine peptidase inhibitor, leupeptin prevented degradation (Moore *et al.*, 1999). The β_2-adrenoceptor was the first identified mammalian GPCR shown to be directed to lysosomes for degradation by ubiquitination (Shenoy *et al.*, 2001), an observation closely followed by that for the C-X-C chemokine receptor type 4 ($CXCR_4$) (Marchese and Benovic, 2001). PARs are sometimes termed one-shot receptors, because of the proteolytic nature of their activation. PAR_2 is also targeted for degradation by lysosomal peptidases by ubiquitination (Jacob *et al.*, 2005). N-CBZ-L-phenylalanyl-L-alanine-diazomethylketone, an inhibitor of lysosomal cysteine peptidases, cathepsins B and L prevents degradation of PAR_2. However, there are GPCRs that are trafficked to lysosomes without the need for modification by ubiquitin moieties. Although, agonists induce ubiquitination of the δ-opioid receptor, this ubiquitination is not an absolute requirement for lysosomal targeting, as a lysine-less mutant of the δ-opioid receptor is still efficiently trafficked to lysosomes and degraded by lysosomal serine peptidases (Tsao and von Zastrow, 2000; Tanowitz and Von Zastrow, 2002). However, further studies have indicated that ubiquitination does have a subtle role in the regulation of δ-opioid receptors. Ubiquitination, although not affecting trafficking to lysosomes does alter the rate at which the δ-opioid receptor is degraded in lysosomes (Hislop *et al.*, 2009). A similar role for ubiquitination is observed for the μ-opioid receptor. It is sequences in the C-terminal tail of the μ-opioid receptor that direct its trafficking to lysosomes, whereas ubiquitination of lysine residues in the first intracellular loop promote transfer of internalized μ-opioid receptors from the limiting endosome membrane to lumen thereby facilitating degradation (Hislop *et al.*, 2011). The receptor for calcitonin gene-related peptide (CGRP) is an unusual heterodimeric GPCR, comprising the GPCR, calcitonin receptor-like receptor (CLR) and a single transmembrane protein, receptor activity-modifying protein 1 (RAMP1) (McLatchie *et al.*, 1998). Following sus-

tained activation with CGRP, CLR•RAMP1 traffics to lysosomes and is degraded by lysosomal peptidases (Kuwasako *et al.*, 2000; Cottrell *et al.*, 2007). The identity of the lysosomal peptidases degrading CLR and RAMP1 is less clear, as an inhibitor cocktail blocking serine, aspartic and cysteine peptidases was used. However, CGRP does not induce ubiquitination of CLR or RAMP1 (Cottrell *et al.*, 2007). Although there are known roles for lysosomal peptidases in the immune system, in generating or destroying antigenic peptides and in trafficking of growth factor receptors [reviewed in Muller *et al.* (2012)], the functional consequences of preventing the degradation of GPCRs in lysosomes remain to be determined.

Proteolytic removal of ubiquitin regulates GPCR-dependent trafficking and signalling

The identity of many of the E3 ligases responsible for facilitating the addition of ubiquitin moieties to GPCRs are now known and are summarized elsewhere (Hislop and von Zastrow, 2011). However, unlike the E3 ligases, fewer USPs responsible for the de-ubiquitination of GPCRs have been identified. USPs play an important role in the regulation of GPCRs by ubiquitination, opposing the action of E3 ligases by removing ubiquitin molecules. This proteolysis is not only important for regulating GPCR signalling, but is also critical for maintaining the cellular pools of ubiquitin, which is critical for many other processes including regulation of transcription, cell-cycle control, DNA damage responses, apoptosis and the immune response [reviewed in Malynn and Ma (2010); Ramaekers and Wouters (2011); Vucic *et al.* (2011); Hammond-Martel *et al.* (2012); Starostina and Kipreos (2012)]. Studies have shown that under basal conditions PAR_1 is ubiquitinated at the cell-surface and that this ubiquitination promotes cell-surface retention (Wolfe *et al.*, 2007). Following proteolytic activation by thrombin, PAR_1 is de-ubiquitinated by unidentified USPs, a process that facilitates internalization to endosomes (Wolfe *et al.*, 2007). $CXCR_7$, a recycling GPCR is also ubiquitinated under basal conditions. After activation induced by the stromal-derived factor CXCL12, $CXCR_7$ is reversibly de-ubiquitinated by an unidentified USP, before trafficking back to the cell-surface (Canals *et al.*, 2012). Unusually, κ-opioid receptors are polyubiquitinated following agonist stimulation. These polyubiquitin chains are subsequently removed by CylD protein, a USP that specifically cleaves Lys-63 ubiquitin chains. After removal of the ubiquitin chains, κ-opioid receptors are degraded in lysosomes (Li *et al.*, 2008). However, other than down-regulation of the κ-opioid receptor, the functional consequences of this polyubiquitination have yet to be elucidated. β_2-Adrenoceptors are regulated by USP33 and USP20, which act in a coordinated fashion to regulate the β_2-adrenoceptor recycling and resensitization (Berthouze *et al.*, 2009). Similarly, ubiquitinated adenosine A_{2A} receptors are de-ubiquitinated by USP4, promoting trafficking back to the cell-surface (Milojevic *et al.*, 2006). In contrast, the proteolytic activities of associated molecule with the SH3 domain of signal-transducing adaptor molecule de-ubiquitinating peptidase (AMSH) and USP8 are required to promote entry of PAR_2 to lysosomes (Hasdemir *et al.*, 2009). AMSH and USP8 also regulate the proteolytic down-regulation of the δ-opioid receptor, but in contrast to PAR_2, their activities do not affect the trafficking δ-opioid receptors to lysosomes (Hislop *et al.*, 2009). Expression of dominant negative mutants of AMSH and USP8 caused accumulation of PAR_2 in endosomes, but had no effect on endosomal mitogenic signalling, indicating that de-ubiquitination does not regulate association with β-arrestins (Hasdemir *et al.*, 2009). The effect of deubiquitination on the mitogenic signalling of other GPCRs has not been examined. However, ubiquitination of $CXCR_4$ by atrophin-interacting protein 4 is reported to enhance $CXCR_4$-mediated ERK1/2 activation (Malik *et al.*, 2012). Although only a few USPs that regulate ubiquitin dynamics for GPCRs have been identified to date, it is clear that de-ubiquitination by peptidases plays a different role for each GPCR, in regulating trafficking and signalling.

The cytosolic proteins, β-arrestin 1 and 2 are key regulators of GPCR signalling and internalization [reviewed in Moore *et al.* (2007); Shenoy and Lefkowitz (2011)] and may be ubiquitinated following agonist stimulation. The first observation of β-arrestin ubiquitination occurred following activation of the β_2-adrenoceptor and is regulated by the E3 ligase, murine double minute 2 (Mdm2) (Shenoy *et al.*, 2001). A later study showed that de-ubiquitination of β-arrestin triggers its release from β_2-adrenoceptors and the V_2 vasopressin receptors (Shenoy and Lefkowitz, 2003). GPCRs may be classed on their association with β-arrestins. Class A receptors bind only β-arrestin 2 and with low affinity, whereas class B receptors bind both β-arrestin 1 and 2 with similar affinities and for prolonged periods (Oakley *et al.*, 2000). When a β-arrestin-ubiquitin fusion protein was overexpressed in cells expressing the β_2-adrenoceptor, the receptor associated with the β-arrestin–ubiquitin fusion protein for prolonged periods, changing the β_2-adrenoceptor from a class A to a class B GPCR (Shenoy and Lefkowitz, 2003). Consistent with this observation and the fact that β-arrestins can mediate GPCR-induced ERK1/2 activation (Luttrell *et al.*, 1999), sustained ubiquitination of β-arrestins results in prolonged activation of ERK1/2 by agonists of β_2-adrenoceptors and V_2 vasopressin receptors (Shenoy *et al.*, 2007) (Figure 3, panel B). The ubiquitination state of β-arrestins is reciprocally regulated by Mdm2 and USP33, with the actions of USP33 promoting the disassembly of endosomal signalling complexes terminating ERK signalling (Shenoy *et al.*, 2009).

There are additional ubiquitin-like modifying proteins, such as small ubiquitin-like modifier 1 (SUMO-1). Upon activation of the β_2-adrenoceptor, β-arrestins are modified by the E2-modifying enzyme Ubc9, in a process termed sumoylation (Wyatt *et al.*, 2011). Although it is known that this sumoylation promotes the internalization of the β_2-adrenoceptor, the identity of the SUMO-specific peptidase and the functional consequences of the desumoylation are not yet known.

Endosomal proteolysis regulates the recycling and resensitization of GPCRs

SP and CGRP are neuropeptides expressed by primary sensory neurons (Lundberg *et al.*, 1985). Noxious stimuli and trauma

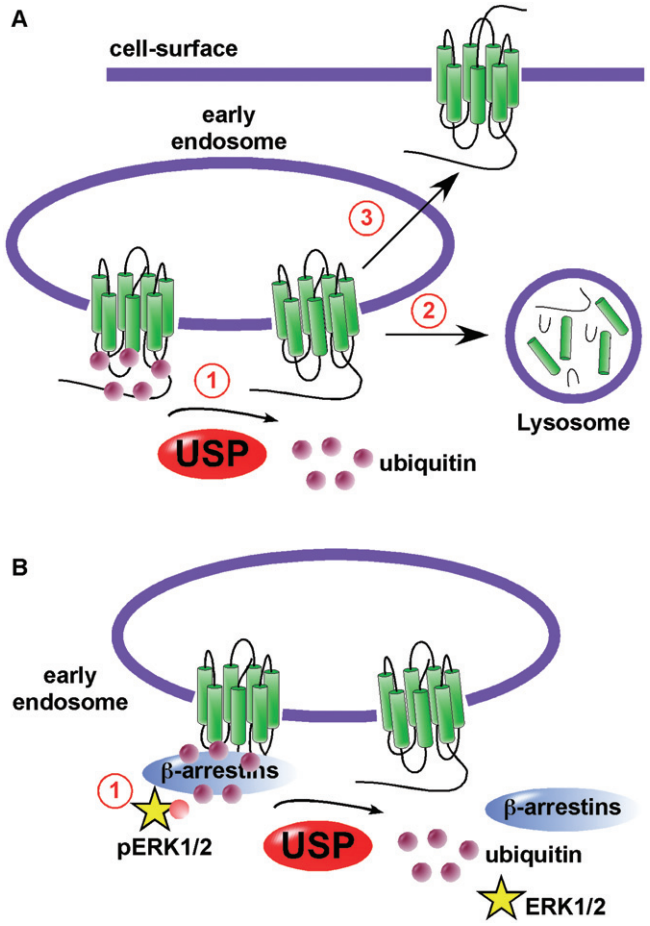

Figure 3

USPs promote lysosomal degradation and recycling of GPCRs. (A) (1) USPs cleave ubiquitin molecules from GPCRs to (2) promote entry in lysosomes and degradation by lysosomal peptidases or (3) recycling of GPCRs to the cell-surface mediating resensitization of signalling. (B) Ubiquitination of β-arrestins promotes GPCR-mediated phosphorylation of extracellular-regulated PK 1 and 2. (2) De-ubiquitination of β-arrestins by USPs destabilizes the GPCR•β-arrestin complex to terminate ERK1/2 activation.

can lead to release of CGRP and SP from primary sensory neurons. The central release of CGRP and SP facilitates nociceptive transmission (Kuraishi *et al.*, 1988; Kawamura *et al.*, 1989; Pedersen-Bjergaard *et al.*, 1991), whereas peripheral release mediates neurogenic inflammation, which is characterized by neutrophil infiltration, oedema and vasodilatation (McDonald, 1988; McDonald *et al.*, 1988). CGRP, a potent vasodilator (Brain *et al.*, 1985) is also implicated in the pathogenesis of migraine [reviewed in Raddant and Russo, (2011); Moore and Salvatore (2012)].

Following activation, many GPCRs internalize with β-arrestins to early endosomes. In order for a GPCR to recycle back to the cell-surface, β-arrestins must be released. Until recently, the molecular mechanisms that initiate the release of β-arrestins from neuropeptide GPCRs, such as the NK_1 receptor and CLR•RAMP1 were poorly defined. NK_1 receptors and CLR•RAMP1 are both class B GPCRs, having sustained interactions with β-arrestins (Oakley *et al.*, 2000; Hilairet *et al.*, 2001). Similar to the mechanisms that operate at the cell-surface, NK_1 receptors and CLR•RAMP1 are regulated by the membrane-anchored peptidase, ECE-1 present in endosomes (Figure 4) (Padilla *et al.*, 2007; Roosterman *et al.*, 2007). SP and CGRP are substrates for the endosomal peptidase, ECE-1, but only at endosomal pH (Johnson *et al.*, 1999; Padilla *et al.*, 2007; Roosterman *et al.*, 2007). As NK_1 receptors and CLR•RAMP1 are trafficked through the endosomal system, vacuolar type H^+-ATPases pump protons inside the vesicles lowering the pH of endosomes (Forgac *et al.*, 1983; Cain *et al.*, 1989). Acidification has two effects: firstly, the affinity of SP and CGRP for their respective receptors is lowered, and secondly, SP and CGRP become substrates for ECE-1 (Padilla *et al.*, 2007; Roosterman *et al.*, 2007). ECE-1 cleaves SP and CGRP into inactive metabolites that can no longer interact with their GPCRs. β-Arrestins are then released and NK_1 receptors and CLR•RAMP1 are free to recycle back to the cell-surface (Padilla *et al.*, 2007; Roosterman *et al.*, 2007). Initial experiments were carried out using transfected cell lines, but further studies have shown that ECE-1-dependent cleavage of these neuropeptides regulates GPCR trafficking both in cells that endogenously express these receptors and in *in vivo* models. For example, ECE-1 regulates the resensitization of SP-induced plasma extravasation both in mice and rats, indicating the ECE-1 regulates NK_1 receptors in endothelial cells (Roosterman *et al.*, 2007; Cattaruzza *et al.*, 2009). ECE-1 also regulates the trafficking of NK_1 receptors in primary myenteric neurons (Pelayo *et al.*, 2011). More recently, we have shown that ECE-1 regulates resensitization of CGRP-induced cAMP generation in primary mesenteric artery smooth muscle cells (McNeish *et al.*, 2012). We also demonstrated that ECE-1 inhibition prevents the resensitization of CGRP-induced relaxation in rat mesenteric resistance arteries (McNeish *et al.*, 2012). This ECE–1-dependent regulation is not confined to NK_1 receptors and CLR•RAMP1, as other GPCRs are also regulated by this mechanism. Somatostatin-14 and –28 are inhibitory peptides exhibiting broad endocrine, exocrine and neuronal functions, such as the suppression of growth hormone secretion and the inhibition of pancreatic and gastrointestinal hormone release [reviewed in Olias *et al.* (2004)]. Somatostatin-14 and –28 exert their biological effects via activation of somatostatin receptors (sst receptors), which are expressed throughout the CNS and endocrine and immune systems. Sst receptors are also found at particularly high densities in many neuroendocrine tumours (Reubi *et al.*, 1987a; 1987b; 1987c). This high density of sst receptors allows imaging of tumours using a radiolabelled analogue of somatostatin called octreotide, a process termed sst scintigraphy (Lamberts *et al.*, 1990). The sst_2 receptor is regulated by ECE-1 following stimulation with somatostatin-14, but not by octreotide, reflecting the ability of ECE-1 to cleave somatostatin-14, but not octreotide (Roosterman *et al.*, 2008). A similar agonist-dependent trafficking was observed in studies with the corticotropin-releasing factor receptor 1 (CRF_1). CRF_1 receptors have two known agonists, corticotropin-releasing factor (CRF) and urocortin 1 (Ucn1). ECE-1 cleaves both peptides at endosonal pH, but only cleaves Unc1 at a residue critical for receptor binding (Hasdemir *et al.*, 2012). At low agonist concentrations (30 nM), both Ucn1- and CRF-mediated intracellular calcium mobilization are dependent

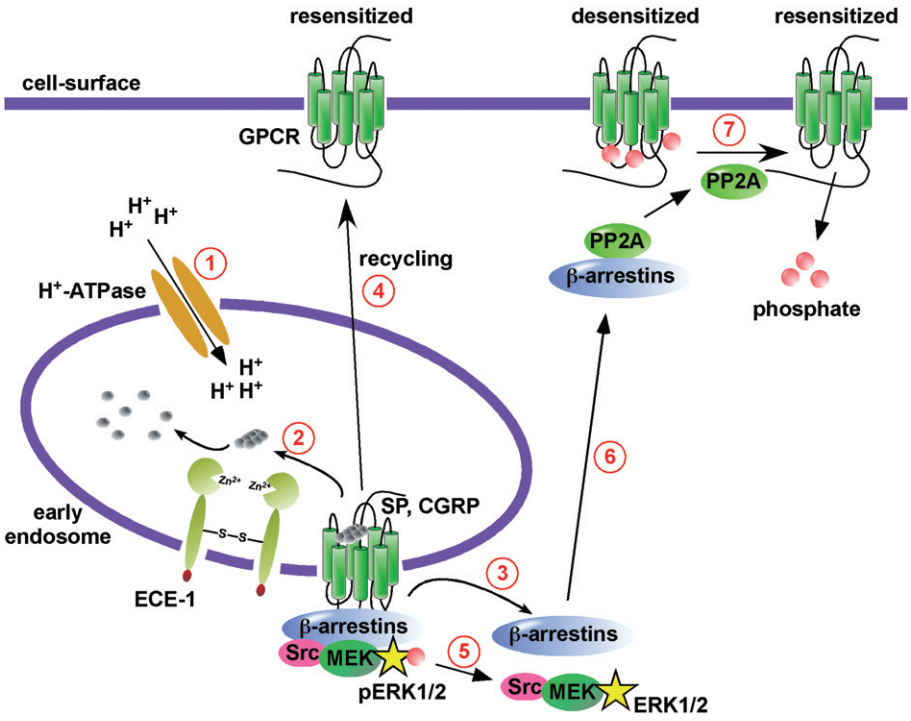

Figure 4

Endosomal peptidases promote GPCR recycling and resensitization. (1) Vacuolar-type H^+-ATPases pump protons (H^+) into vesicles, acidifying early endosomes. (2) Peptide agonists such as SP and CGRP have reduced affinity for their respective GPCRs. SP and CGRP become substrates for the endosomal peptidase, ECE-1 at low pH and are hydrolysed to inactive metabolites. (3) β-Arrestins dissociate from the GPCR, returning to the cytosol. (4) The GPCR, free from β-arrestins then recycles back to the cell-surface to mediate resensitization. (5) Certain GPCRs (e.g. neurokinin-1 receptor) signal from endosomes in a β-arrestin-dependent mechanism, phosphorylating extracellular-regulated PKs 1 and 2 (pERK1/2). ECE-1 promoted dissociation of β-arrestins terminates ERK1/2 activation. (6) β-Arrestins can recruit protein phosphatases such as protein phosphatase 2A (PP2A) to desensitized GPCRs at the cell-surface. (7) PP2A activity dephosphorylates cell-surface located GPCRs promoting resensitization.

on ECE-1 activity; however, at high concentrations (100 nM), CRF-mediated intracellular calcium mobilization and CRF_1 receptor recycling and resensitization cease to be ECE-1-dependent. This loss of ECE–1-dependent trafficking perhaps reflects a mechanism to mediate distinct CRF_1 receptor trafficking and signalling, at higher concentrations of agonist (Hasdemir *et al.*, 2012). Neurotensin is also a substrate for ECE-1 at endosomal pH (Johnson *et al.*, 1999) and mediates intestinal inflammation and cell proliferation through activation of the neurotensin 1 receptor (NTS_1) (Castagliuolo *et al.*, 1999; Brun *et al.*, 2005). Endosomal ECE-1 activity promotes degradation of neurotensin and recycling of NTS_1 receptors (Law *et al.*, 2012).

Not all peptide-activated GPCRs are regulated by ECE-1. Studies have shown that although ECE-1 degrades bradykinin, ECE-1 does not regulate the recycling and resensitization of B_2 receptors (Padilla *et al.*, 2007). This is because of the nature of the interaction of B_2 receptors with β-arrestins, B_2 receptors only exhibit a transient interact with β-arrestins (Simaan *et al.*, 2005). Thus, it is not only whether ECE-1 cleaves the agonist at endosomal pH that determines if ECE-1 regulates the GPCR, but also the duration of the association of the GPCR with β-arrestins. Moreover, agonists must be substrates for ECE-1 at endosomal pH in order for the GPCR to be regulated by ECE-1. Although angiotensin I is a substrate for ECE-1, angiotensin II is not (Johnson *et al.*, 1999; Padilla *et al.*, 2007) and ECE-1 does not regulate the recycling and resensitization of the AT_2 receptors (Padilla *et al.*, 2007).

An additional role for ECE-1-mediated cleavage of SP emerged following the observation that resensitization of SP-induced intracellular calcium mobilization precedes recycling of the NK_1 receptor (Bennett *et al.*, 2002; 2005; Murphy *et al.*, 2011). It is known that agonist-unoccupied GPCRs are desensitized by phosphorylation of serine and threonine residues by the second messenger kinases, PKA and PKC (Roth *et al.*, 1991; Pitcher *et al.*, 1992; Dery *et al.*, 2001). This second messenger kinase-dependent phosphorylation desensitizes the GPCR without causing internalization. In the case of the NK_1 receptor, it is the dephosphorylation of cell-surface located, desensitized receptors that mediates resensitization of SP-induced signalling (Figure 4) (Murphy *et al.*, 2011). ECE-1-dependent cleavage of SP releases β-arrestins from the endosomal GPCR complex. β-Arrestins then facilitate the recruitment of protein phosphatase 2A (PP2A), a known regulator of GPCRs (Pitcher *et al.*, 1995) to the cell-surface, where PP2A dephosphorylates NK_1 receptors to promote resensitization (Murphy *et al.*, 2011). It is not currently known if the same or a similar mechanism controls the resensitization of other ECE-1-regulated GPCRs. Further, it is not yet known whether other endosomal peptidases regulate the trafficking of other peptide-activated GPCRs. Peptidases may represent a therapeutic target, whereby inhibitors of peptidases would

prevent recycling and resensitization of GPCRs to prevent uncontrolled GPCR-mediated signalling that contributes to disease.

Proteolysis regulates endosomal signalling of GPCRs

In contrast to their role at the cell-surface in terminating G protein-dependent signalling, an additional function of β-arrestins is the recruitment of the signalling molecules to GPCRs in early endosomes. The signalling apparatus recruited to GPCRs by β-arrestins, serves to initiate a second wave of signalling that is distinct from that initiated at the cell-surface (Luttrell *et al.*, 1999). This endosome-based signalling is a relatively new area of investigation and has been recently reviewed (von Zastrow and Sorkin, 2007; Murphy *et al.*, 2009). For certain GPCRs, proteolysis of ligands by peptidases located in endosomes, serves as the molecular switch that determines the duration of this β–arrestin-dependent signalling. At the endosome surface, β-arrestins act as a scaffold to aid formation of mitogenic signalling complexes that include signalling proteins, such as Raf-1, MAPK kinases and ERK (Daaka *et al.*, 1998; DeFea *et al.*, 2000). The stability of these so-called signalosomes is dependent on the sustained interaction of β-arrestins with GPCRs in endosomes. Thus, for NK_1 receptors, ECE–1-dependent hydrolysis of SP regulates the stability of the NK_1 receptor•β-arrestin interaction and thus, the duration of SP-induced β–arrestin-dependent ERK activity (Cottrell *et al.*, 2009). This endosome-derived ERK1/2 activation up-regulates and phosphorylates the nuclear death receptor, Nur77 via a mechanism that requires β-arrestins, Raf-1, MAPK kinase 2 and ERK2 (Castro-Obregon *et al.*, 2004). Inhibition of ECE-1, which prevents the dissociation of NK_1 receptors and β-arrestins (Roosterman *et al.*, 2007), causes a sustained activation of ERK1/2, increased phosphorylation of Nur77, promoting cell death (Cottrell *et al.*, 2009). This mechanism also operates in cultured primary myenteric neurons and in intact animals (Cottrell *et al.*, 2009; Pelayo *et al.*, 2011). In mice, intraplantar injection of capsaicin, an activator of transient receptor potential vanilloid ion channel 1, promotes the release of SP in the dorsal horn to cause internalization of NK_1 receptors and activation of ERK1/2 (Mantyh *et al.*, 1995; Kawasaki *et al.*, 2004). An intrathecal injection of the highly selective ECE-1 inhibitor SM-19712 (Umekawa *et al.*, 2000) promoted a more sustained ERK1/2 activation following injection of capsaicin (Cottrell *et al.*, 2009). In contrast to SP-induced ERK1/2 activation, neurotensin-induced ERK1/2 activation is attenuated by ECE-1 inhibition, indicating that the recycling of NTS_1 receptors promotes ERK1/2 activation (Law *et al.*, 2012). ECE-1 inhibition similarly attenuated JNK activation, but promoted NF-κB activation and IL-8 secretion (Law *et al.*, 2012).

Concluding remarks

It has long been known that peptidases present on the cell-surface regulate GPCR activation by generating or destroying bioactive peptides, and that many GPCRs are ultimately degraded by peptidases present in lysosomes. However, it is now apparent that proteolysis also regulates other aspects of GPCRs, including trafficking through the endocytic system and signalling from endosomes. Intracellular proteolysis of peptidic agonists by peptidases present in endosomes, not only controls the recycling and resensitization of GPCRs, but also regulates the signalling from internalized GPCRs in endosomes. Ubiquitination of GPCRs targets certain GPCRs to lysosomes, but de-ubiquitination performed by USPs is required for efficient delivery of GPCRs to lysosomes, promoting GPCR down-regulation. For other GPCRs, USPs regulate the recycling to the cell-surface and thus, resensitization of signalling. Finally, USPs act to regulate the ubiquitination of β-arrestins and downstream mitogenic signalling cascades. ACE inhibitors, such as captopril have been successfully used to treat hypertension for many years and offer hope that other peptidase inhibitors could also be used to treat disease. Inhibitors of endosomal peptidases, such as ECE-1 could be used to regulate the trafficking of CGRP and SP receptors. ECE-1 inhibitors, by preventing the recycling of CGRP receptors, could prevent sustained CGRP signalling implicated in migraine. Inhibitors of ADAM peptidases, responsible for the generation of ligands causing transactivation of ErbB receptors, may represent a new therapy to prevent proliferation of cells and thereby attenuate cancer growth. The signalling pathways arising from internalized GPCRs are distinct from pathways initiated at the cell-surface and have unique cellular consequences. Inhibitors of USPs and endosomal peptidases have been shown to regulate this signalling and thus inhibitors of these peptidases may be useful tools to regulate the signalling of internalized GPCRs. Thus, the discovery of these new peptidase-driven mechanisms of GPCR regulation opens up a new line of potential peptidase inhibitor therapies to treat GPCR-mediated diseases. A key factor in the design of these peptidase inhibitors will be targeting them to the cellular compartments where they will have the required effects.

Acknowledgements

Supported by British Heart Foundation Fellowship to G.S.C (FS/08/017/25027).

Conflict of interest

The author declares no conflict of interest.

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