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REVIEW

The relationship between the MMP system, adrenoceptors and phosphoprotein phosphatases

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The MMPs and their inhibitors [tissue inhibitor of MMPs (TIMPs)] form the mainstay of extracellular matrix homeostasis. They are expressed in response to numerous stimuli including cytokines and GPCR activation. This review highlights the importance of adrenoceptors and phosphoprotein phosphatases (PPP) in regulating MMPs in the cardiovascular system, which may help explain some of the beneficial effects of targeting the adrenoceptor system in tissue remodelling and will establish emerging crosstalk between these three systems. Although α- and β-adrenoceptor activation increases MMP but decreases TIMP expression, MMPs are implicated in the growth stimulatory effects of adrenoceptor activation through transactivation of epidermal growth factor receptor. Furthermore, they have recently been found to catalyse the proteolysis of β-adrenoceptors and modulate vascular tone. While the mechanisms underpinning these effects are not well defined, reversible protein phosphorylation by kinases and phosphatases may be key. In particular, PPP (Ser/Thr phosphatases) are not only critical in resensitization and internalization of adrenoceptors but also modulate MMP expression. The interrelationship is complex as isoprenaline (ISO) inhibits okadaic acid [phosphoprotein phosphatase type 1/phosphoprotein phosphatase type 2A (PP2A) inhibitor]-mediated MMP expression. While this may be simply due to its ability to transiently increase PP2A activity, there is evidence for MMP-9 that ISO prevents okadaic acid-mediated expression of MMP-9 through a β-arrestin, NF-κB-dependent pathway, which is abolished by knock-down of PP2A. It is essential that crosstalk between MMPs, adrenoceptors and PPP are investigated further as it will provide important insight into how adrenoceptors modulate cardiovascular remodelling, and may identify new targets for pharmacological manipulation of the MMP system.

Abbreviations

ADAM, A disintegrin and metalloproteinase; AP-1, activator protein-1; CARV, carvedilol; CREB, cAMP responsive element-binding protein; ECM, extracellular matrix; EGFR, epidermal growth factor receptor; GRK, GPCR kinase; HB-EGF, heparin-binding EGF-like growth factor; IκB, inhibitor of κB; I-1, inhibitor-1; I-2, inhibitor-2; ISO, isoprenaline; MI, myocardial infarction; NA, noradrenaline; PEA3, polyomavirus enhancer-binding activator-3; PI3K, phosphoinositide 3 kinase; PLA2, phospholipase A2; PP, protein phosphatase; PPM, metal-dependent phosphatase; PPP, phosphoprotein phosphatase; PRAZ, prazosin; ROS, reactive oxygen species; Sp1, specificity protein 1

Introduction

MMPs belong to a large family of zinc- and calcium-dependent endopeptidases capable of degrading extracellular matrix (ECM) proteins. They are characterized by three conserved histidines (HEXXHXXGXXH) in the zinc-binding motif of their active centre, along with a conserved methionine, which forms a Met-turn (Bode *et al.*, 1993). Of the 28 MMP genes currently identified, 24 are found in man (Sternlicht and Werb, 2001).

The classification of MMPs has moved towards a combined approach based on domain structure, cellular localization and activation mechanism (Ra and Parks, 2007). Under this system, MMPs are divided into two main groups, secreted and membrane-integrated/anchored. Secreted MMPs are subclassified on the basis of having a minimal domain (MMP-7, -26), a gelatin-binding domain (MMP-2, -9) or a typical domain structure (MMP-1, -3, -8, -10, -11, -12, -13, -18, -19, -20, -22, -27, -28). The membrane-associated MMPs are also subdivided into type I membrane MMPs (MMP-14, -15, -16, and -24), type II membrane MMPs (MMP-23) and glycosylphosphatidyl inositol-anchored MMPs (MMP-17, -25).

As the ECM is both a space-filling material and a bioactive molecule modulating cell adhesion, migration, proliferation and survival (Werb, 1997; Bowers *et al.*, 2010), the functional consequences of its degradation by MMPs under physiological and pathophysiological conditions is clear. However, their action is not limited to degradation of the ECM, as they are involved in activation and processing of TNF-α, Fas ligand and adhesion molecules (Gearing *et al.*, 1994; Mitsiades *et al.*, 2001; Vaisar *et al.*, 2009), as well as activation/cleavage of cell surface receptors, including adrenoceptors (Prenzel *et al.*, 1999; Rodrigues *et al.*, 2010). As such, there has been great interest in developing MMP inhibitors. However, their clinical utility has been disappointing, and a growing body of evidence indicates that targeting signal transduction pathways may be a better approach to limit MMP-mediated tissue remodelling (Overall and Lopez-Otin, 2002).

In this review, we provide a brief overview of MMP regulation and go on to consider how adrenoceptors and protein phosphatases (PP) modulate MMP abundance, primarily in the cardiovascular system, and discuss potential crosstalk between these systems.

MMP regulation

MMP abundance is tightly controlled through multiple mechanisms including transcription/epigenetic, compartmentalization, zymogen activation and inhibition by endogenous tissue inhibitors of MMPs (TIMPs), and is briefly reviewed below.

Transcriptional regulation

MMPs frequently share commonality in their cis-acting elements. Several contain a TATA box, a proximal activator protein-1 (AP-1) binding motif and often a polyomavirus enhancer-binding activator-3 (PEA3) binding site (e.g. MMP-1, -3, -7, -9, -10, -12, -13, -19 and -26), while others have a TATA box but no proximal AP-1 binding site (e.g. MMP-8, MMP-11 and MMP-21), or have neither (e.g. MMP-2, MMP-14 and MMP-28) (Yan and Boyd, 2006; Vincenti and Brinckerhoff, 2007). While this is useful in stratifying MMPs, it is an oversimplification as most promoters contain binding motifs for multiple transcription factors. A case in hand is the MMP-9 promoter (Figure 1), which has a TATA box and several consensus sequences for NF-κB, AP-1, TGF-β inhibitory element, specificity protein 1 (Sp1)/GC and PEA3 (Sato and Seiki, 1993; Sato *et al.*, 1993; Van den Steen *et al.*, 2002). Indeed, there are five putative AP-1 binding sites and two conserved NF-κB binding sites, one for p65 and another for p50 (Han *et al.*, 2001); these are key for regulation by cytokines. It also has a variable length microsatellite $(CA)_{13-27}$ repeat, the length of which is directly related to promoter activity in diabetic and stroke patients (Peters *et al.*, 1999; Maeda *et al.*, 2001), although this has not been noted in healthy volunteers (Demacq *et al.*, 2008). Nevertheless, an *in vitro* study clearly demonstrates that shortening of the microsatellite sequence inhibits MMP-9 expression in human lung adenocarcinoma cells (Huang *et al.*, 2003).

While transcriptional regulation of MMP-9 may appear quite straightforward, many cis-regulatory elements function in a synergistic manner. Early studies found that the proximal AP-1 binding site cooperates with the NF-κB (–600 bp) and Sp1 binding sites to mediate 12-O-tetradecanoyl-phorbol-13-acetate (PMA) and TNF-α-induced MMP-9 promoter activity (Sato and Seiki, 1993; Bond *et al.*, 1998). More recently, a ternary co-activator complex comprised of cAMP responsive element-binding protein (CREB)/p300, co-activator-associated arginine methyltransferase 1 and a p160 steroid receptor co-activator (SRC) family member; the glucocorti-

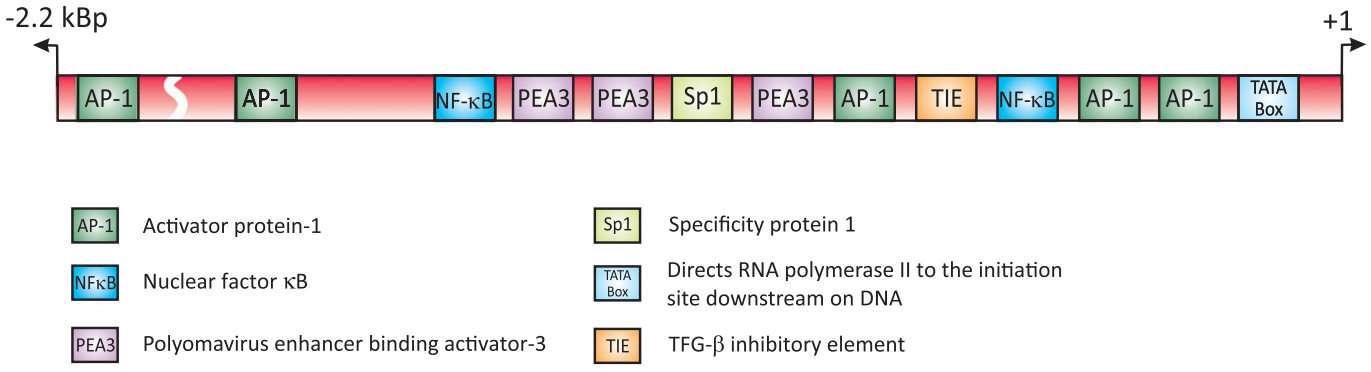

Figure 1

A schematic representation of the transcription factor binding sites in the human MMP-9 promoter (based upon data from Han *et al.*, 2001; Huhtala *et al.*, 1991; Sato *et al.*, 1993; Song *et al.*, 2008; Van den Steen *et al.*, 2002).

coid receptor interacting protein-1 has also been established to modulate MMP-9 gene expression following PMA stimulation (Zhao and Benveniste, 2008).

Compartmentalization

Targeted degradation by MMPs can be achieved through compartmentalization within the cell, at the cell surface or within the ECM. For instance, release of MMPs stored in neutrophils and macrophages (Belaaouaj *et al.*, 1995; Ardi *et al.*, 2007; Rosell *et al.*, 2008) limits their action to sites of injury following cellular activation. Secreted MMPs may also be localized to the cell surface through binding to docking receptors, like CD44 (hyaluronan receptor), which facilitates MMP-9-mediated proteolysis and activation of latent TGF-β in keratinocytes (Yu and Stamenkovic, 2000). Additionally, co-localization of MMP-9 with CD44 and α4β1 integrin is implicated in leukaemic B cell migration and survival (Redondo-Munoz *et al.*, 2008; 2010).

Zymogen activation

Once synthesized, MMPs are either constitutively released or secreted in response to a variety of signals as a latent or proMMP form (Sternlicht and Werb, 2001). ProMMPs are activated either intracellularly or extracellularly through proteolytic or oxidative cleavage of a 'cysteine switch' in their prodomain. MMP-11 (stromelysin-3) was the first MMP found to be activated intracellularly by furin, a Golgi-associated protease, as part of the secretory pathway (Pei and Weiss, 1995). Extracellular activation of zymogens is best documented for proMMP-2 (*in vitro*), which is activated at the cell surface through the cooperative action of MMP-14 [membrane type 1 (MT1)-MMP] and TIMP-2 in a 1:1:1 stoichiometry (Imai *et al.*, 1996; Knauper *et al.*, 1996; Sato *et al.*, 1996). Although data pertaining to *in vivo* activation of MMPs are sparse, the *in vitro* data are consistent with the observation that only proMMP-2 is found in TIMP-2 knock out (KO) mice (Wang *et al.*, 2000). The role of MMP-14 is less clear, as there is no change in proMMP-2 activation in MMP-14 KO mice (Ruangpanit *et al.*, 2002). This phenomenon is not limited to MMP-14, as MMP-2 and MMP-3 can activate proMMP-9 (Ramos-DeSimone *et al.*, 1999; Toth *et al.*, 2003), as can elastase, trypsin and mast cell chymases (Ferry *et al.*, 1997; Descamps *et al.*, 2004; Tchougounova *et al.*, 2005). ProMMPs can also be activated non-enzymatically through oxidation of thiol groups in their prodomain by reactive oxygen species (ROS) which promotes autolytic cleavage (Nelson and Melendez, 2004).

Endogenous inhibitors

MMP activity is fine-tuned by interaction with endogenous inhibitors such as α_2-macroglobulin and TIMPs. α_2-Macroglobulin is an endopeptidase inhibitor, which contains a bait region that is cleaved by proteases including metalloproteases. Once cleaved, it undergoes a conformational change trapping the MMP (Nagase *et al.*, 1994). While this is the principal inhibitor, TIMPs likely have the greatest influence on MMP activity.

To date, four TIMPs have been identified (TIMP-1, -2, -3, -4), and inhibit MMP activity by binding in a 1:1 stoichiometry with varying affinity and specificity. TIMP-1 preferentially inhibits MMP-3, MMP-7 and MMP-9, while TIMP-2 inhibits MMP-2. TIMP-3 is bound to the ECM and has the broadest inhibitory spectrum, inhibiting not only MMP-2 and MMP-9, but also members of the A disintegrin and metalloproteinases (ADAMs), and ADAMs with thrombospondin motif (ADAMTS) families. Finally, TIMP-4 has a predilection for MMP-14 (MT1-MMP) and MMP-2. The TIMPs also exhibit differential binding to the latent isoforms of gelatinases, as TIMP-2, -3 and -4 can bind to proMMP-2, while TIMP-1 and -3 bind to proMMP-9 (Wang *et al.*, 2000; Brew and Nagase, 2010). Mechanistically, the N-terminal domain of the TIMPs interact with the catalytic domain of the MMPs to inhibit their activity, while the C-terminal binds to proMMP-2 and -9 via their haemopexin domain, which stabilizes the inhibitor complex. Although many of the functional consequences of TIMPs are attributed to modulation of MMP activty, new evidence indicates that they can influence cell differentiation, growth, migration and angiogenesis in their own right (reviewed in Brew and Nagase, 2010).

Adrenoceptors and the MMP system

Since adrenoceptors were first classified into α- and β-adrenoceptor subtypes by Ahlquist in 1948 (Ahlquist, 1948), and shown to mediate the effects of sympathetic nerve stimulation, they have been further subdivided into α_1-, α_2- and β-adrenoceptors based on their pharmacological profile, physiological function and tissue location. Each of these adrenoceptor families currently contains three subtypes: the α_{1A}-, α_{1B}-, α_{1D}-; α_{2A}-, α_{2B}-, α_{2C}- and β_1-, β_2-, β_3-adrenoceptors. Adrenoceptors underlie a plethora of physiological functions ranging from metabolic effects to vasoconstriction and immune modulation (Brodde, 1991; Civantos and Aleixandre de, 2001; Oberbeck, 2006). Nonetheless, it is well established that prolonged exposure to catecholamines contributes to vascular and cardiac remodelling in experimental models and in man. Moreover, evidence suggests that adrenoceptors may modulate this process via the MMP system.

α-Adrenoceptor activation (Table 1)

Several lines of evidence support the role of α-adrenoceptors in controlling MMP abundance (Figure 2). Firstly, noradrenaline (NA) increases cardiac MMP-2 activity and mRNA expression, and decreases TIMP-1 and TIMP-2 expression in rat and mouse models of NA-induced cardiac remodelling (Briest *et al.*, 2001; 2004; Meier *et al.*, 2007). This effect is attributed to its α-agonist activity as the response is attenuated by prazosin (PRAZ) but not metoprolol (Briest *et al.*, 2004). Secondly, long-term exposure to α_1-adrenoceptor antibodies, which activate the receptor, increases cardiac MMP-2 expression and interstitial collagen deposition in rat (Zhou *et al.*, 2005). Data from our laboratory extend these studies to show that phenylephrine induces MMP-9 promoter activity in ECV304 cells, although the response is refractory to PRAZ (Song *et al.*, 2006). This is interesting as it may indicate the presence of α_{1L}-adrenoceptors, which have a low affinity for PRAZ (Ford *et al.*, 1997), or may reflect the low potency of α-blockers in certain tissues due to a tissue-specific property of the receptors (Argyle and McGrath, 2000).

α_2-Adrenoceptors are also implicated in modulation of the MMP system as brimonidine, an α_2-adrenoceptor agonist,

Table 1

α-Adrenoceptors and the MMP system

Receptor	Drug	MMP/TIMP	Tissue/Model	Notes	Authors
α_1	Bunazosin	MMP-2, -3, -9 ↔	Monkey ciliary muscle cells	6 h	(Akaishi *et al.*, 2004)
α_1	Phenylephrine	MMP-9 ↑	ECV304 cells	24 h	(Song *et al.*, 2006)
α_1	Noradrenaline; prazosin	MMP-2, TIMP-2 ↑ (NA); ↔ (NA + PRAZ)	Rat ventricular myocardium	3 to 4 days	(Briest *et al.*, 2004)
α_1	Doxazosin	MMP-2, -3, -9 ↔; TIMP-1 ↔	Human mesangial cells + macrophage medium	1, 3 days	(Pawluczyk *et al.*, 2006)
α_1	Prazosin	MMP-9 ↑	Rat skeletal muscle	7 days	(Van Gieson and Skalak, 2001)
α_1	Bunazosin	MMP-3 activity ↑, MMP-3 mRNA ↔; MMP-1, -2 ↔; TIMP-1, -3 ↓	Rat conjunctival tissue, human keratinocytes and fibroblasts	Daily, 2 weeks	(Ito *et al.*, 2006)
α_1	6-Hydroxydopamine; prazosin	TIMP-1 ↓; Liver fibrosis ↓	CCl_4-induced liver fibrosis in rats	6 weeks	(Dubuisson *et al.*, 2002)
α_1	Doxazosin	TIMP-2/MMP-2 ratio ↔	Aortic-banded rats	10 weeks	(Perlini *et al.*, 2005)
α_1	Terazosin	proMMP-2, MMP-2 ↑; TIMP-1,-2 ↔	Rat ventral prostate	120 days	(Mitropoulos *et al.*, 2007)
α_1	Anti- α_1-adrenoceptor antibody	MMP-2 ↑	Mouse heart	1 year	(Zhou *et al.*, 2005)
α_2	Brimonidine	proMMP-9 expression ↑, activity ↔; TIMP-4 ↓; proMMP-1, -2, -3, and -24 ↔	Ciliary body smooth muscle cells	1 and 3 days	(Ooi *et al.*, 2009)
α_2	Brimonidine	MMP-3 ↑; MMP-1, -2 ↔; TIMP-1, -3 ↓	Rat conjunctival tissue, human keratinocytes and fibroblasts	Daily, 2 weeks	(Ito *et al.*, 2006)
$\alpha_{1/2}$	Phenoxybenzamine	MMP-2, -9 ↓; TIMP-1 ↑	Pig vein	2 weeks	(Chung *et al.*, 2005)
ND	Noradrenaline	TIMP-1, -3, MMP-2 ↑	Mouse heart	4 h; 1, 3 days	(Meier *et al.*, 2007)
ND	Noradrenaline	MMP-2, TIMP-2 ↑	Rat heart	3, 4, 7 days	(Briest *et al.*, 2001)

NA, noradrenaline; ND, not determined; PRAZ, prazosin; ↑, up-regulation; ↔, no effect; ↓, down-regulation.

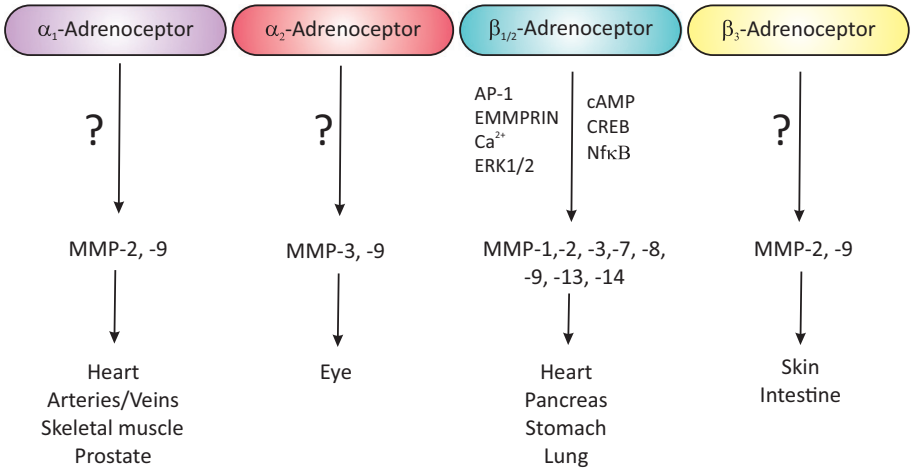

Figure 2

A diagrammatic summarizing the interrelationship between adrenoceptor subtypes and MMP expression, detailing tissues/organs where this has been noted along with specifics of the signal transduction pathways. EMMPRIN, extracellular matrix metalloproteinase inducer.

increases MMP-3 and proMMP-9 expression in rat conjunctival and human ciliary body smooth muscle cells, while attenuating TIMP-1, -3 and -4 expression (Ito *et al.*, 2006; Ooi *et al.*, 2009). However, it has no effect on MMP-1, MMP-2 or TIMP-2 expression in rat conjunctival tissue (Ito *et al.*, 2006).

α-Adrenoceptor antagonists (Table 1)

Other studies have focussed on the effects of α-adrenoceptor antagonists on the MMP system, albeit with little consensus. For instance, when jugular vein grafts are pharmacologically relaxed with phenoxybenzamine (non-selective α-adrenoceptor antagonist), MMP-9 and pro/active MMP-2 expression are reduced compared to mechanically distended vessels (Chung *et al.*, 2005). Juxtaposed to this, the α_1-adrenoceptor antagonists, PRAZ and terazosin, increase MMP-9 and pro/active MMP-2 expression in rat skeletal muscle and rat ventral prostate respectively (Van Gieson and Skalak, 2001; Mitropoulos *et al.*, 2007). Yet others report that doxazosin and bunazosin do not affect MMP-2, MMP-3 or MMP-9 abundance in human mesangial cells, monkey ciliary muscle cells or conjunctival tissue (Akaishi *et al.*, 2004; Ito *et al.*, 2006; Pawluczyk *et al.*, 2006).

It is also unclear how α_1-adrenoceptor antagonists alter TIMPs. While they have been found to not alter TIMP-1 or TIMP-2 abundance in rat ventral prostate (Mitropoulos *et al.*, 2007) or in conditioned medium from human mesangial cells or monkey ciliary muscle cells (Akaishi *et al.*, 2004; Pawluczyk *et al.*, 2006), bunazosin decreases TIMP-1 and -3 expression in rat conjunctival tissue (Ito *et al.*, 2006) and phenoxybenzamine increases TIMP-1 in porcine jugular vein (Chung *et al.*, 2005).

Although these studies provide clear evidence that α-adrenoceptor agonists and antagonists modulate MMP and TIMP expression, clarity over the net effect remains to be established. The lack of consensus may reflect differences in the regulation of individual MMP and TIMP family members, species-/tissue-/cell line-specific properties, differences in the model employed (unstimulated vs. stimulated conditions) or duration of exposure to the α-adrenoceptor modulating agents and time point at which the effects are studied (Table 1).

β-adrenoceptor activation and the MMP system (Table 2)

Non-selective β-adrenoceptor activation (isoprenaline). Chronic administration of isoprenaline (ISO) to rats not only causes extensive tissue remodelling but also increases cardiac-specific expression of MMP-1, MMP-2 and MMP-9 (Miura *et al.*, 2003; Hori *et al.*, 2009; 2011). Kizaki *et al.* report that ISO increases MMP-2 but not MMP-9 mRNA expression in rats (Kizaki *et al.*, 2005). While the reason for the lack of effect on MMP-9 in the latter study is not clear, it may reflect differences in exposure time to ISO (Table 2) or may represent its effects on the spatial dispersion of MMP-9 in the left ventricle and septum, but not the right ventricle (Cheng *et al.*, 2009).

These effects could be mediated through a plethora of cell types present in the complex milieu of the heart, such as inflammatory cell infiltrates, fibroblasts and cardiomyocytes. Indeed, ISO potentiates LPS-induced MMP-1 and MMP-9 expression in human monocytes and U937 cells, an effect which is abrogated by β_1- but not β_2- or α-adrenoceptor antagonism (Speidl *et al.*, 2004). Nevertheless, Coker *et al.* demonstrated that ISO can increase MMP-2 and MMP-14 (MT1-MMP) abundance in conditioned media from isolated ventricular cardiomyocytes (Coker *et al.*, 2001), a finding corroborated by Menon *et al.* who showed that ISO increases MMP-2 and TIMP-1 but decreases TIMP-2 mRNA expression in isolated adult ventricular cardiomyocytes (Menon *et al.*, 2005).

β_1-Adrenoceptors clearly have a role in this, as cardiac over-expression of β_1-adrenoceptors in mice is associated with development of cardiac hypertrophy and increased expression of MMP-1, proMMP-2 and MMP-13 in the short term (5 months). By 12 months of age, the animals have overt cardiac dilation and fibrosis, and MMP-2 and MMP-14 (MT1-MMP)

Table 2

β-Adrenoceptors and the MMP system

Receptor	Drug	MMP/TIMP	Tissue/Model	Notes	Authors
β_1	Adrenaline; noradrenaline; phenoxybenzamine; doxazosin; yohimbine; propranolol; metoprolol; butoxamine	MMP-1, -9 ↑ (ADN/NA); ↔ (ADN/NA + PROP); ↔ (ADN/NA + MET); TIMP-1 ↑ (ADN/NA)	PBMC, macrophages, U937 cells	48 h	(Speidl *et al.*, 2004)
β_1	Noradrenaline; metoprolol	MMP-2, TIMP-2 ↑ (NA); ↔ (NA + MET)	Rat ventricular myocardium	3 to 4 days	(Briest *et al.*, 2004)
β_1	Metoprolol	MMP-1 ↓; MMP-13 ↔; TIMP-1 ↓; TIMP-2 ↔	TGF-β1 TG mice	6 weeks	(Seeland *et al.*, 2009)
β_1	Metoprolol	MMP-1 ↓	Rabbit vulnerable plaque model (local transfection of p53)	12 weeks	(Liang *et al.*, 2009)
β_1	–	5 months: MMP-1, -13 and proMMP-2 ↑; 12 months: proMMP-2 and MMP-14, TIMP-2 ↑	Transgenic mice over-expressing β_1-adrenoceptors	5, 12 months	(Seeland *et al.*, 2007)
β_2	Clenbuterol (agonist)	MMP-9 ↓; proMMP-2 ↑	Mouse heart	20 h	(Patiyal and Katoch, 2005)
β_2	Formoterol (agonist)	MMP-9 ↓; MMP-2 ↔	Cadmium induced acute pulmonary inflammation in rats	24 h	(Zhang *et al.*, 2010b)
β_2	Salbutamol (agonist)	MMP-9 ↑; TIMP-1, -2 (*in vitro* only) ↓	Distal lung epithelial cells and bronchioalveolar lavage fluid	4 days	(O'Kane *et al.*, 2009)
β_2	–	MMP	β_2-AR over-expressing transgenic mice		(Xu *et al.*, 2011)
β_3	SAR150640 (agonist)	MMP-2, -9 ↔; MMP-2, -9 ↓ (+ LPS)	LPS-stimulated human myometrial samples	48 h	(Lirussi *et al.*, 2010)
β_3	Adrenaline; propranolol; SR-59230A; phentolamine	MMP-2 ↓ (ADN); ↓↓ (ADN + PROP); ↔ (ADN + SR); ↓ (ADN + PHEN)	Murine dermal fibroblasts	24 h	(Romana-Souza *et al.*, 2011)
$\beta_{1/2}$	Isoprenaline	MMP-2 ↓ (1 nM); ↑ (1 μM)	Rat adrenal medulla endothelial cells	3 h	(Papadimitriou *et al.*, 2001)
$\beta_{1/2}$	Isoprenaline	MMP-9 ↑	ECV304 cells	4 h	(Song *et al.*, 2006)
$\beta_{1/2}$	Isoprenaline	MMP-2 ↑; MMP-9 ↔; MMP-14 ↑	Porcine left ventricular myocytes	6 h	(Coker *et al.*, 2001)
$\beta_{1/2}$	Noradrenaline; propranolol	MMP-2, -9 ↑ (NA); ↔ (NA + PROP)	Nasopharyngeal carcinoma tumour cell lines	6 and 12 h	(Yang *et al.*, 2006)
$\beta_{1/2}$	Isoprenaline; propranolol; ICI 118,551	MMP-7 ↑ (ISO); ↔ (ISO + PROP/ICI)	Human gastric cancer cell lines	12 and 24 h	(Shi *et al.*, 2010)
$\beta_{1/2}$	Noradrenaline; propranolol	MMP-2 ↑ (NA); ↔ (NA + PROP)	Human umbilical vein endothelial cells (HUVECs)	18 h	(Lamy *et al.*, 2010)
$\beta_{1/2}$	Isoprenaline	MMP-2, -3, -7 and -9 ↔	Human hepatic cancer cell lines	24 h	(Lodewyks *et al.*, 2011)
$\beta_{1/2}$	Isoprenaline	MMP-2, TIMP-1 ↑; TIMP-2 ↓; MMP-9 ↔	Rat ventricular myocytes	24 h	(Menon *et al.*, 2005)
$\beta_{1/2}$	Isoprenaline	MMP-9 ↑ (okadaic acid); ↔ (okadaic acid +I SO)	Murine embryonic fibroblasts, adult human cardiac ventricular fibroblasts	24 h	(Rietz *et al.*, 2012)
$\beta_{1/2}$	Noradrenaline; propranolol; prazosin	MMP-2 ↑ (NA + PRAZ); EMMPRIN expression ↑ (NA + PRAZ), ↔ (NA + PROP)	Adult rat ventricular myocytes	24 h	(Siwik *et al.*, 2008)
$\beta_{1/2}$	Noradrenaline; propranolol	MMP-2, -9 ↑ (NA); ↔ (NA + PROP)	Human pancreatic cancer cell lines	24 h	(Guo *et al.*, 2009)

Table 2

Continued

Receptor	Drug	MMP/TIMP	Tissue/Model	Notes	Authors
$\beta_{1/2}$	Isoprenaline	MMP-2 ↑; MMP-9 ↔	Rat cardiac ventricles	4 days	(Kizaki *et al.*, 2005)
$\beta_{1/2}$	Isoprenaline	MMP-2 ↑; TIMP ↔	Spontaneously hypertensive rats	4–5 days	(Veliotes *et al.*, 2010)
$\beta_{1/2}$	Isoprenaline	MMP-9 mRNA LV ↔, septum ↑, RV ↔; MMP-9 expression LV ↑, septum ↑, RV ↓	Rat heart	10 days	(Cheng *et al.*, 2009)
$\beta_{1/2}$	Isoprenaline; spironolactone	MMP-2 ↑ (ISO); ↔ (ISO + spironolactone)	Rat myocardial ventricle	14 days	(Hori *et al.*, 2011)
$\beta_{1/2}$	Isoprenaline; doxycycline	MMP-2, -9 ↑ (ISO); ↔ (ISO + DOX)	Rat myocardial ventricles	14 days	(Hori *et al.*, 2009)
$\beta_{1/2}$	Propranolol	proMMP-9 ↓	PMA stimulated-brain tumour- derived medulloblastoma cells	18 h	(Annabi *et al.*, 2010)
$\beta_{1/2}$	Propranolol	proMMP-9 ↓; proMMP-2 ↔	PMA stimulated-human brain microvascular endothelial cells	18 h	(Annabi *et al.*, 2009)
$\beta_{1/2}$	Propranolol; ICI 118,551; Metoprolol	MMP-2, -9 ↓ (PROP; ICI; MET)	Human pancreatic cancer cells	24 h	(Zhang *et al.*, 2010a)
$\beta_{1/2}$	Propranolol	MMP-8, -9 mRNA ↑ (MI), ↑↑ (MI + PROP); MMP-9 activity ↑ (MI), ↔ (MI + PROP); MMP-2 ↓ (MI), ↓↓ (MI + PROP)	Rat model of myocardial infarction (MI)	6–72 h	(Deten *et al.*, 2003)
$\beta_{1/2}$	Propranolol	MMP-2, -9 ↑ (day 14; stress); ↑ (day 7, 14 stress + PROP)	Chronically stressed mice, wound healing	7 and 14 days	(Romana-Souza *et al.*, 2010)
$\beta_{1/2}$	Timolol; betaxolol; carteolol	TIMP-1, -2, -3 ↑ (TIMO); MMP-3, -2, -11↓(TIMO); TIMP-2 ↑ (BET); MMP-3 ↑ (CART)	Rat conjunctival tissue	Daily, 2 weeks	(Ito *et al.*, 2006)
$\beta_{1/2}$	Propranolol	MMP-2, -9 ↓	Human gastric cancer cells		(Liao *et al.*, 2010)
$\beta_{1/2}$	Carvedilol; metoprolol; metoprolol + bunazosin	MMP-8, -13 ↑ (all); TIMP-1 ↔ (all)	Rat ligated heart model after myocardial infarction	4 weeks	(Zhuang *et al.*, 2009)
$\beta_{1/2}$	Propranolol	TIMP-2/MMP-2 ratio ↑	Aortic-banded rats	10 weeks	(Perlini *et al.*, 2005)
α/β	Carvedilol; probucol; propranolol; prazosin	MMP-2, -9 ↑ (ATH); ↓ (ATH + CARV/PROB); MMP-2, -9 ↑ (TNF-α); MMP-2, -9 ↓ (TNF-α + CARV/PROB but not PROP/PRAZ)	Rabbit model of atherosclerosis (ATH); human aortic smooth muscle cells.	21 days; 18 h drugs prior TNF-α (24 h)	(Wu *et al.*, 2007)
ND	Carvedilol; metoprolol	MMP-3, -8, -9, -14 ↑, TIMP-4; MMP-8 ↓ (+ CARV)	Murine coxsackie virus myocarditis	9 days	(Pauschinger *et al.*, 2005)
ND	Carvedilol	MMP-2 ↑; ↔ (+ CARV); TIMP-1 ↓; ↑ (+ CARV)	Daunorubicin-induced cardiotoxicity and nephrotoxicity in rats	41 days	(Arozal *et al.*, 2010)
ND	Carvedilol	proMMP-9 ↓; TIMP-1 ↔	Heart failure patients plasma	4, 12 weeks	(Song *et al.*, 2006)
ND	Carvedilol	MMP-9 ↑; ↓ (+ CARV)	Dilated cardiomyopathic rat	90 days	(Chua *et al.*, 2008)
ND	Carvedilol	MMP-1, -3, -9, TNF-α ↑, NA ↑; MMP-1, -3, -9, TNF-α ↓ (+ CARV)	Idiopathic dilated cardiomyopathy patients plasma	6 months	(Ohtsuka *et al.*, 2003)

ADN, adrenaline; ATH, atherosclerosis; BET, betaxolol; CARV, Carvedilol; CART, carteolol; DOX, doxycycline; EMMPRIN, extracellular matrix metalloproteinase inducer; ICI, ICI-118 551; ISO, isoprenaline; LV, left ventricle; MET, metoprolol; NA, noradrenaline; ND, not determined; PBMC, peripheral blood mononuclear cells PHEN, phentolamine; PRAZ, prazosin; PROB, probucol; PROP, propranolol; RV, right ventricle; SR, SR-59230A; TIMO, timolol; ↑, up-regulation; ↑↑, augmented up-regulation; ↔, no effect; ↓, down-regulation; ↓↓ augmented down-regulation.

expression is increased (Seeland *et al.*, 2007). Moreover, MMP-14 (MT1-MMP) was found to co-localize with MMP-2, which is consistent with its proposed role in activating proMMP-2 (Imai *et al.*, 1996; Knauper *et al.*, 1996; Sato *et al.*, 1996).

It is worth noting that β-adrenoceptor modulation of MMPs is not restricted to the cardiovascular system (Figure 2), as recent studies indicate that NA and ISO increase MMP-2, MMP-7 and MMP-9 abundance in a β-adrenoceptor-dependent manner in human pancreatic and gastric cancer cells (Guo *et al.*, 2009; Shi *et al.*, 2010).

β_2- and β_3-adrenoceptor agonists

The role of β_2-adrenoceptors in MMP modulation has only been reported in a select number of studies (Table 2). In patients with acute respiratory distress syndrome, salbutamol (β_2-agonist) increases MMP-9 activity in bronchioalveolar lavage fluid, and increases MMP-9 but decreases TIMP-1 and -2 expression in distal lung epithelial cells (O'Kane *et al.*, 2009). Similar findings are reported for formoterol (β_2-adrenoceptor agonist) in a rat model of pulmonary inflammation (Zhang *et al.*, 2010b). However, in the mouse heart, clenbuterol (β_2-adrenoceptor agonist) decreases MMP-9 activity (Patiyal and Katoch, 2005). More recently, over-expression of β_2-adrenoceptors has been associated with increased MMP-2 activity and progressive development of ventricular dysfunction in mice. Surprisingly, these effects were largely abrogated by N-acetylcysteine, indicating regulation of MMP-2 by ROS (Xu *et al.*, 2011).

It is only in the last two years that a couple of studies have emerged implicating β_3-adrenoceptor in MMP regulation (Figure 2). SAR150640 (β_3-adrenoceptor agonist) increases MMP-2 and -9 abundance in human myometrial strips (Lirussi *et al.*, 2010), while SR-59230A, a β_3-adrenoceptor antagonist, inhibits adrenaline-induced MMP-2 abundance in skin fibroblasts (Romana-Souza *et al.*, 2011).

β-adrenoceptor antagonists and the MMP system

The clinical benefits of β-adrenoceptor antagonists in heart failure (Packer *et al.*, 1996; Goldstein and Hjalmarson, 1998) are well documented. However, a seminal study by McDonald *et al.* revealed that metoprolol (β_1-adrenoceptor antagonist) could regress established ventricular remodelling in a canine model of myocardial damage, independent of haemodynamic changes (McDonald *et al.*, 1994). This raised the possibility that β-blockers may affect remodelling through effects on the MMP system. Senzaki *et al.* published the first report supporting this hypothesis in 2000, in which they showed that supra-therapeutic doses of atenolol (β_1-adrenoceptor antagonist; 2.8 g·day^{-1} vs. clinical maximum dose of 200 mg·day^{-1}) attenuates the increase in MMP-9 abundance associated with heart failure in pigs (Senzaki *et al.*, 2000). Since this report, several groups have demonstrated that metoprolol abrogates the increase in MMP-2 abundance in an experimental model of myocardial infarction (MI; Table 2) (Bernstein and Tyagi, 2001; Cimmino *et al.*, 2011), and reduces MMP-1 abundance and vessel remodelling in a rabbit model of the vulnerable plaque (Liang *et al.*, 2009). In addition, timolol (non-selective β-antagonist) increases TIMP-1, -2 and -3 expression in conjunctival tissue (Ito *et al.*, 2006). While these effects may represent an anti-fibrotic process, metoprolol increases (normalized) expression of MMP-1 and decreases TIMP-1 abundance in the myocardium, while reducing cardiomyocyte size consistent with an anti-hypertrophic rather than an anti-fibrotic effect in TGF-β1 over-expressing mice (Seeland *et al.*, 2009).

Clinically, in patients with idiopathic dilated cardiomyopathy, 6 months treatment with carvedilol (CARV; α_1- and $\beta_{1/2}$-adrenoceptor antagonist) reduces plasma levels of MMP-1 and -9, but not MMP-3 abundance (Ohtsuka *et al.*, 2003). In keeping with this, short-term (12 weeks) treatment with CARV attenuates the increase in plasma proMMP-9 activity observed in non-CARV-treated heart failure patients (Song *et al.*, 2006). Although not studied, data from an animal study would indicate that the decrease in MMP-9 abundance may be manifest as a decrease in myocardial fibrosis and apoptosis (Chua *et al.*, 2008). CARV also prevents MMP-8 up-regulation in a murine model of viral myocarditis (Pauschinger *et al.*, 2005), and reduces MMP-2 and MMP-9 expression in a rabbit model of atherosclerosis and following exposure of human aortic smooth muscle cells to TNF-α (Wu *et al.*, 2007). Interestingly, in the latter study, effects on MMP-2 and MMP-9 were unaffected by propranolol or PRAZ (Wu *et al.*, 2007). This raises the possibility that the inhibitory effects of CARV on MMP abundance may be related to its antioxidant rather than adrenoceptor blocking properties (Yue *et al.*, 1992); oxidative stress is a potent regulator of MMPs (Siwik *et al.*, 2001; Kameda *et al.*, 2003). In support of this, CARV decreases cardiac expression of MMP-2 and reduces oxidative stress in a rat model of daunorubicin-induced cardiotoxicity (Arozal *et al.*, 2010). While the antioxidant properties of CARV may be important, propranolol, which is not renowned for its antioxidant properties (some of its metabolites are antioxidants, Mak and Weglicki, 2004) decreases MMP-9 and/or MMP-2 abundance in a rat model of MI (Deten *et al.*, 2003), in human gastric cancer cells (Liao *et al.*, 2010) and in human umbilical vein endothelial cells (Lamy *et al.*, 2010). This aside, propranolol differentially abrogates PMA-mediated MMP-9 and MMP-2 expression in human brain endothelial cells (Annabi *et al.*, 2009) and medulloblastoma cells (Annabi *et al.*, 2010), supporting receptor-dependent modulation.

How are β-adrenoceptors coupled to MMPs?

Delineation of the signalling mechanisms coupling β-adrenoceptors to MMPs is not well investigated. However, a cAMP/PKA axis appears to be a key component (Figure 2). For instance, activation of cAMP-dependent protein kinase by dibutyryl-cAMP or forskolin (adenylate cyclase activator) increases MMP-9 and MMP-2 abundance (Liu *et al.*, 2004; Staun-Ram *et al.*, 2004; Pavlovic *et al.*, 2006), while ISO increases MMP-2 expression via cAMP-dependent phosphorylation of CREB in cardiomyocytes and cardiofibroblasts from a phosphoinositide 3 kinase γ (PI3Kγ) knockout model of heart failure (Guo *et al.*, 2010). Likewise, expression of other cAMP-responsive MMPs (MMP-14 and MMP-13) are also increased by ~3- and 12-fold, respectively (Guo *et al.*, 2010), as is TIMP-1 mRNA expression (Liu *et al.*, 2004).

Juxtaposed to this, ISO, forskolin, dibutyryl-cAMP and intracellular Ca^{2+} chelation decrease MMP-2 expression in rat adrenal medullary endothelial cells (Papadimitriou *et al.*,

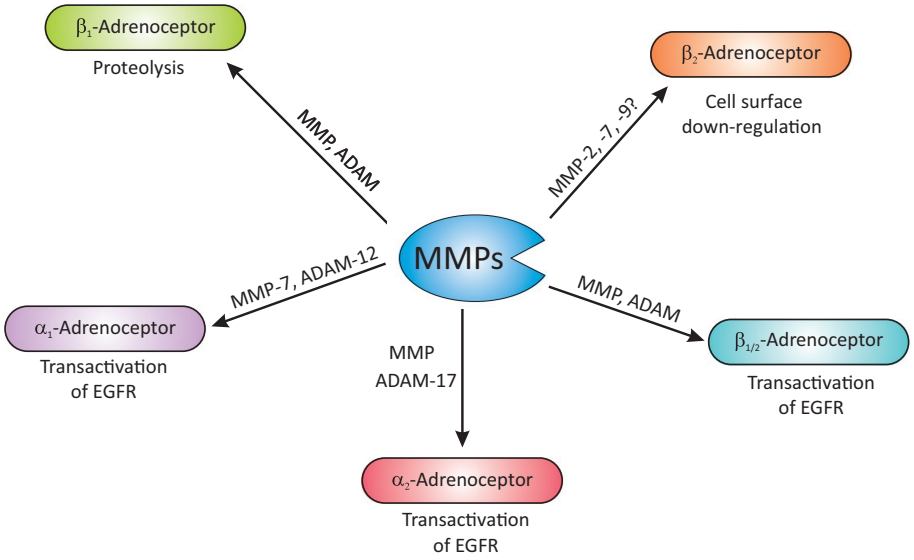

Figure 3

A graphic representation of the central role that MMPs and ADAMs have in the proteolysis of β-adrenoceptor, and in mediating transactivation of EGFR following α- and β-adrenoceptor stimulation via release of HB-EGF (see text for details).

2001). Furthermore, forskolin decreases MMP-9 activity in keratinocytes following exposure to EGF (McCawley *et al.*, 2000), while dibutyryl-cAMP, IBMX and rolipram (phosphodiesterase inhibitors) attenuate LPS-induced transcription of MMP-9 in rat primary astrocytes (Lee *et al.*, 2008). The latter appears to occur independent of PKA involvement, as the response is refractory to H89 and Rp-cAMP (Lee *et al.*, 2008). In keeping with the inhibitory effects of ISO, it decreases okadaic acid-induced MMP-9 expression through a pathway, which is dependent upon an intact NF-κB binding motif, but is not associated with altered inhibitor of κB (IκB) expression in fibroblasts (Rietz *et al.*, 2012). Furthermore, in the absence of okadaic acid, ISO increases MMP-9 expression via a 762-bp region (from 1285-523) of its promoter, which contains consensus sequences for NF-κB and AP-1 (Song *et al.*, 2006). Why this disparity exists requires further investigation. Crosstalk between β-adrenoceptors and MMPs may be indirect, as over-expression of a dominant negative extracellular matrix metalloproteinase inducer prevents β-adrenoceptor-mediated induction of MMP-2 (Siwik *et al.*, 2008). Moreover, aldosterone receptor antagonism also prevents ISO-induced MMP-2 expression, but not that of TIMP in spontaneously hypertensive rats or a rat model of ISO-induced ventricular fibrosis (Veliotes *et al.*, 2010; Hori *et al.*, 2011).

The effects of β-adrenoceptor antagonists on MMP modulation may simply represent blockade of the agonist response, as ICI 118,551 (β_2-adrenoceptor selective antagonist) abrogates ISO-induced MMP-7 expression in an AP-1 dependent manner, via c-Jun interacting with signal transducer and activator of transcription 3 at an AP-1 binding motif in gastric cancer cells (Shi *et al.*, 2010). Alternatively, ICI 118,551 can inhibit MMP-2 and MMP-9 expression in the absence of agonist in human pancreatic cancer cells, which may be mediated through NF-κB, AP-1 and CREB, as their expression is decreased (Zhang *et al.*, 2010a). In support of this, propranolol in addition to decreasing MMP abundance (Deten *et al.*, 2003; Annabi *et al.*, 2010; Liao *et al.*, 2010) also inhibits NF-κB expression (Liao *et al.*, 2010), reduces phosphorylation of IκB (Annabi *et al.*, 2010), inhibits ERK-1/2 signalling (Lamy *et al.*, 2010) and decreases nucleocytoplasmic export of the mRNA-stabilizing factor HuR (Annabi *et al.*, 2009); many of which are well established in regulating the MMP system (Yan and Boyd, 2006). In contrast, CARV, metoprolol and co-administration of metoprolol and bunazosin increase MMP-8 and MMP-13 expression and NF-κB expression in non-infarcted tissue from a rat model of MI (Zhuang *et al.*, 2009). This apparent inconsistency likely reflects complexity and diversity in the regulation of different MMP family members (Yan and Boyd, 2006; Vincenti and Brinckerhoff, 2007).

Other synergism between MMPs and adrenoceptors

MMPs and adrenoceptor cleavage

Although the preceding sections highlight the role of adrenoceptors in modulating MMP abundance, emerging reports now indicate that MMPs are involved in catalysing proteolysis of β_1- and β_2-adrenoceptors (Figure 3). Utilizing an *in vitro* and *in vivo* approach, Hakalahti *et al.* eloquently demonstrated that GM6001 (non-specific MMP and ADAM inhibitor) prevented cleavage of the N-terminus of the β_1-adrenoceptor at Arg^{31} and Leu^{32} and Pro^{52} and Leu^{53} (Hakalahti *et al.*, 2010). In addition, ISO induces proteolysis of the receptor in a time- and concentration-dependent manner, an effect which is mimicked by activation of PKC and adenylate cyclase (Hakalahti *et al.*, 2010). Around the same time, Rodrigues *et al.* discovered that doxycycline

(MMP inhibitor) and EDTA prevented the loss of β_2-adrenoceptors from the plasma membrane of aortic endothelial cells and cardiac micro-vessels from control vessels exposed to plasma from spontaneously hypertensive rats (Rodrigues *et al.*, 2010). Although neither of these studies identified the MMP(s) involved, it could be MMP-2 as its activity is ~4-fold higher in the aorta from spontaneously hypertensive rats compared to normotensive controls; MMP-9 activity is virtually undetectable (Spiers *et al.*, 2005). This paradigm is strengthened by a recent study showing that MMP-2 and NF-κB mediate proteolysis of the extracellular domain of β_2-adrenoceptors in kidney from spontaneously hypertensive rats (Wu and Schmid-Shonbein, 2011). Nevertheless, other MMPs such as MMP-7 and MMP-9 could also be involved as they attenuate vascular tone following intravenous administration in spontaneously hypertensive rats (Rodrigues *et al.*, 2010).

Adrenoceptors, MMPs and transactivation

Catecholamines have important growth regulatory and remodelling effects, which are mediated through activation of the MAPK signalling cascade. Several paradigms have been proffered to explain this link, including canonical GPCR signalling pathway involving activation of ERK1/2 MAPK and adrenoceptor-mediated transactivation of epidermal growth factor receptor (EGFR). The latter is thought to occur via MMP-dependent shedding of heparin-binding EGF-like growth factor (HB-EGF) and subsequent activation of the EGFR (Prenzel *et al.*, 1999), or it may involve intracellular activation of Src and agonist-independent phosphorylation of EGFR (Luttrell *et al.*, 1997). In the case of MMP-dependent transactivation of EGFR, it is both receptor and cell type specific, and involves multiple intermediaries including G_i switching, β-arrestin, free radicals, Src, phospholipase A2 (PLA2), PLC and arachidonic acid metabolites.

Both α- and β-adrenoceptors are associated with transactivation of EGFR (Figure 3). α_1-Adrenoceptor-mediated transactivation has been found to involve ROS generation and proteolytic cleavage by MMPs (e.g. MMP-7) in smooth muscle cells, rat mesenteric arteries and cardiomyocytes (Hao *et al.*, 2004; 2006; Zhang *et al.*, 2004; Li *et al.*, 2011). Interstingly, Hao *et al.* found that doxycyclin (MMP inhibitor) reduced systolic blood pressure and HB-EGFR shedding in spontaneously hypertensive rats, implicating EGFR transactivation in α_1-adrenoceptor-mediated regulation of vascular tone and hypertrophy (Hao *et al.*, 2004). The effects on vascular tone may be mediated through either regulation of mitochondrial ATP synthesis by PI3K (Nagareddy *et al.*, 2009) or via Src-independent but PI3K- and ERK1/2 MAPK-dependent signalling (Ulu *et al.*, 2010). In keeping with these studies, MMP-mediated transactivation of EGFR following α_1-adrenoceptor activation in a murine hypothalamic neuronal cell line involves PKC, ERK1/2 MAPK, ROS and Src (Shah *et al.*, 2006). α_2-Adrenoceptors can also initiate ERK1/2 signalling via MMP-dependent transactivation of EGFR through PLA2 and arachidonic in a renal tubular cell line (Cussac *et al.*, 2002). However, in α_2-adrenoceptor transfected PC12 cells, arachidonic release and PLC, but not PLA2, is involved in transactivation of EGFR (Karkoulias *et al.*, 2006).

With regard to β_1-adrenoceptors, ERK1/2 signalling is instigated via a cascade involving GPCR kinase (GRK) (GRK5 and GRK6), β-arrestin-dependent activation of Src and MMP/HB-EGF-mediated EGFR transactivation (Noma *et al.*, 2007). Interestingly, this does not require the participation of G-proteins. The role of β-arrestin is interesting as it maintains formation of a β_1-adrenoceptor/EGFR complex that retains ERK1/2 in the cytosol, while activation of EGFR by EGF directs ERK1/2 to the nucleus (Tilley *et al.*, 2009). As the authors point out, this may represent an innovative mechanism by which β_1-adrenoceptors may educe diverse cellular responses through modulation of EGFR signalling. Surprisingly, when a library of β-adrenoceptor antagonists was studied, alprenolol and CARV also elicited G-protein-independent but β-arrestin-dependent β_1-adrenoceptor transactivation of EGFR (Kim *et al.*, 2008), which may account for their effects on the MMP system.

The process may be more complex as β-adrenoceptors can switch from being coupled to G_s to G_i in response to PKA-mediated receptor phosphorylation (Daaka *et al.*, 1997; Martin *et al.*, 2004). In particular, at low β-agonist (ISO) concentrations, β_2-adrenoceptors are activated resulting in β-arrestin-2-dependent activation of Src, which in turn activates the EGFR. However, at high agonist concentrations, β_1-adrenoceptors transactivate EGFR via a G_i-protein-mediated pathway involving β-arrestin, MMP and HB-EGF in astrocytes (Du *et al.*, 2010). In addition, β_2-adrenoceptors can transactivate EGFR through a pathway similar to that of β_1-adrenoceptors, as it involves Src, MMP and HB-EGF in cardiac fibroblasts (Kim *et al.*, 2002), but is independent of MMP/HB-EGF in COS7 cells (Drube *et al.*, 2006). Other members of the metzincin superfamily, to which MMPs belong, such as ADAM-10, -12 and -17 (Figure 3), are also implicated in α- and β-adrenoceptor-mediated transactivation of EGFR via cleavage of EGF precursors (Prenzel *et al.*, 1999; Asakura *et al.*, 2002; Yan *et al.*, 2002; Schafer *et al.*, 2004; Chen *et al.*, 2006; Karkoulias *et al.*, 2006; Hakalahti *et al.*, 2010; Peng *et al.*, 2010).

Interrelationship between phosphoprotein phosphatases, MMPs and adrenoceptors

Reversible protein phosphorylation mediated by protein kinases and phosphatases is probably the single most important event regulating cell function. While the role of kinases has been extensively studied, that of PP is less well investigated. Nonetheless, it is now apparent that they are equally important in mediating cell signalling, apoptosis, growth, migration and adhesion. Within this group of enzymes, the serine/threonine PP form a major subfamily, which can be subdivided into phosphoprotein phosphatases (PPP), metal-dependent protein phosphatases (PPM) and aspartate-based PP. The PPP subfamily has seven family members, PP1, PP2A, PP2B (calcineurin), PP4, PP5, PP6 and PP7. Of these, phosphoprotein phosphatase type I (PP1) and type 2A (PP2A) catalyse approximately 90% of all serine/threonine dephosphorylation reactions (Haystead *et al.*, 1989). Their activity is tightly regulated by several endogenous inhibitors including inhibitor-1 (I-1), inhibitor-2 (I-2) and dopamine- and cAMP-

regulated phosphoprotein of 32 kDa, which inhibit PP1, while I-1 of PP2A and I-2 of PP2A (I_2^{PP2A}) modulate PP2A (for review, see Shi, 2009).

PPP and MMPs

PPP have an emerging role in tissue remodelling through modulation of the MMP system, and via regulation of adrenoceptor signalling. It was first noted in 1997 by Neumann *et al*. that PP1 activity is increased in patients with end-stage heart failure, and it was proposed that this may well be causally related to disease development, or may exacerbate 'loss of function' to inotropic agents (Neumann *et al*., 1997). This premise was strengthened by data from murine models in which over-expression of PP1 or PP2A depressed cardiac function and caused ventricular remodelling (Carr *et al*., 2002; Gergs *et al*., 2004). However, this may be PPP subtype specific, as no change in total calcineurin activity (protein phosphatase 3; PP2B) is found in tissue from patients with compensated left ventricular hypertrophy (Grammer *et al*., 2006). While the authors did not note any change in MMP-2/MMP29 or TIMP-1–4 abundance, this may be because calcineurin A_β is up-regulated while calcineurin B_α is down-regulated.

Despite this, the consensus from other studies utilizing pharmacological agents to inhibit PPP supports their involvement in MMP and TIMP regulation. Holladay *et al*. published one of the earliest reports showing that okadaic acid, a protein phosphatase 1 and 2A inhibitor (Millward *et al*., 1999), increases MMP-3 (stromelysin-1) expression in murine keratinocytes (Holladay *et al*., 1992). This has been confirmed and extended by others to include collagenase, interstitial collagenase (rat homologue of collagenase-3/MMP-13), MMP-13 and MMP-9 in fibroblasts and chondrocytes (Westermarck *et al*., 1994; 2000; Grumbles *et al*., 1996; Rietz *et al*., 2012). Interestingly, these MMPs have a TATA box, proximal AP-1 and PEA3 binding sites in their promoters (Yan and Boyd, 2006). The pharmacological studies are strengthened by data from a knock-down study demonstrating that silencing of PPM1A (PPM family member) increases proMMP-9 but decreases TIMP-2 expression in extravillous trophoblasts (Zhang *et al*., 2009). There is little evidence implicating calcineurin or PP2B in regulation of the MMP system as cyclosporine but not tacrolimus increases TIMP-1 expression, and neither affect MMP-9 expression (Esposito *et al*., 2004).

So what mechanism(s) contribute to the increase in MMP abundance following PPP inhibition? While this question remains largely unexplored, it may simply reflect maintenance of the phosphorylation and hence activation state of signalling pathways upstream of transcriptional regulation of the MMPs. For instance, PP2A mediates dephosphorylation of MAPK kinase 1 and ERK family kinases (Gomez and Cohen, 1991; Garcia *et al*., 2002; Zhou *et al*., 2002), which are associated with MMP and TIMP regulation (Yan and Boyd, 2006). More specifically, Westermarck's group found that okadaic acid-induced expression of MMP-3 is mediated through transactivation of AP-1 complexes containing c-Jun and JunB in HT-1080 cells (Westermarck *et al*., 1994). However, the process may be more complex than this as recent work from our group shows that okadaic acid increases MMP-9 abundance in fibroblasts through p38-MAPK, and requires intact AP-1 transcription factor binding sites, albeit independently of PP2A inhibition, as knockout of PP2A by siRNA did not alter the response (Rietz *et al*., 2012). This does not rule out the involvement of other PPP, as okadaic acid also inhibits phosphoprotein phosphatase 1 and 5, and recombinant PP4, leading to increased phosphorylation of numerous proteins including Cdc25, histone H1, phosphorylase kinase, PKA, PKB, PKC, IκB kinases and cyclin-dependent kinases (Fujiki *et al*., 1991; Hastie and Cohen, 1998; Dean *et al*., 2001; Janssens and Goris, 2001). This aside, data from transgenic mice over-expressing dominant negative c-Jun (TAM-67) would indicate that c-Jun is not involved in regulating MMP-3 expression (Thompson *et al*., 2002).

PP and adrenoceptors

Receptor phosphorylation and dephosphorylation play a key role in the regulation of GPCR function. This is best exemplified by the β_2-adrenoceptor, which is phosphorylated by GRK2 and PKA, resulting in β-arrestin recruitment to the receptor complex. Binding of β-arrestin sterically prevents the re-coupling of the G-protein to the receptor, and marks the receptor for internalization via clathrin-coated pits (Rockman *et al*., 2002; Delom and Fessart, 2011). Once internalized, receptors are either dephosphorylated (resensitization) by protein phosphatase 2A in the early endosome (Krueger *et al*., 1997) prior to recycling back to the plasma membrane, or undergo ubiquitin-mediated degradation (Figure 4A) (Shenoy *et al*., 2001). Therefore, it is not surprising that ISO increases serine/threonine PP activity in rat heart (Boknik *et al*., 2000), and PP2A activity in ventricular cardiomyocytes, keratinocytes and mouse fibroblasts (Pullar *et al*., 2003; De Arcangelis *et al*., 2008; Rietz *et al*., 2012). A functional consequence of this increase in PP2A activity is enhanced cardiac contractility due to altered myofilament sensitivity to Ca^{2+} (Wijnker *et al*., 2011).

PPP have other effects on adrenoceptor function that are probably independent of endosomal dephosphorylation. In Rat-1 fibroblasts expressing α_{1b}-adrenoceptors, okadaic acid increases receptor phosphorylation and attenuates G-protein coupling, independent of effects on intracellular calcium and inositol trisphosphate formation (Alcantara-Hernandez *et al*., 2000). While this initial report indicates that PKC is responsible for the increase in receptor phosphorylation, this is now questionable, as a follow-up study found the response to be refractory to staurosporine, a PKC inhibitor (Alcantara-Hernandez and Garcia-Sainz, 2005). Nevertheless, the latter study established that receptor phosphorylation and desensitization involve recrutiment of PI3K and Akt/PKB to the receptor. In a similar vein, β_2-adrenoceptors are desensitized via PI3Kγ-dependent activation of I_2^{PP2A}, thereby inhibiting PP2A-mediated dephosphorylation of the receptor at the plasma membrane, thus driving the system towards desensitization and internalization of the receptor (Figure 4B) (Vasudevan *et al*., 2011). PP2A is also involved in a feedback mechanism to protect against or limit sympathetic overdrive. In this scenario, high concentrations of ISO activate PP2A, and limit PKA-dependent contraction of ventricular cardiomyocytes from β_2-adrenoceptor knockout mice. This is associated with recruitment of PP2A to a PKA/A kinase-anchoring complex (De Arcangelis *et al*., 2008).

Several other studies show that PKA and β-adrenoceptor activation inhibit PP1 activity, albeit through activation/

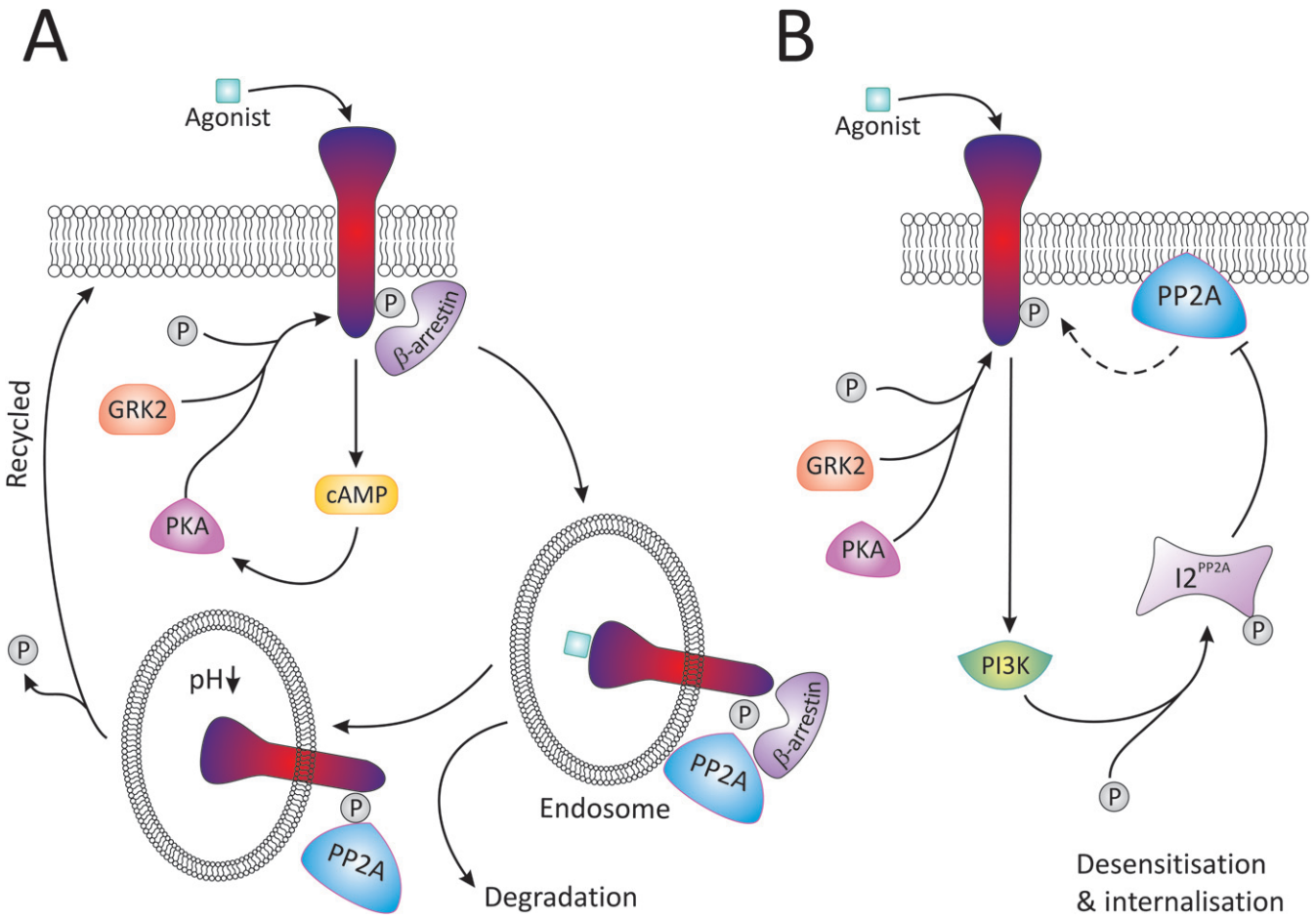

Figure 4

A summary of the role PP2A and its inhibitor $I2^{PP2A}$ have in β-adrenoceptor resensitization (A) and desensitization (B). (A) Following agonist, cAMP levels are elevated and either PKA or GRK2 phosphorylate the receptor permitting binding of β-arrestin. The receptor β-arrestin complex undergoes sequestration into the endosome; following acidification, β-arrestin is released and PP2A dephosphorylates the receptor. As a result of this, the receptor is resensitized and may be recycled back to the plasma membrane. (B) Alternatively, agonist binding and receptor phosphorylation can lead to PI3K activation, which phosphorylates $I2^{PP2A}$ thereby inhibiting PP2A-mediated dephosphorylation of the receptor at the plasma membrane, thus driving the system towards desensitization and internalization of the receptor.

phosphorylation of I-1 (Neumann *et al.*, 1991; Carr *et al.*, 2002; Gupta *et al.*, 2002; El-Armouche *et al.*, 2003; El-Armouche and Eschenhagen, 2009). However, it is unclear if this and the decrease in expression of I-1 in cardiac tissue from patients with heart failure (El-Armouche *et al.*, 2008) and following exposure to ISO in hearts from rats (Boknik *et al.*, 2000; El-Armouche *et al.*, 2007) are beneficial. For example, cardiac over-expression of a constitutively active form of I-1 clearly protects against ISO-induced apoptosis by increasing B-cell lymphoma 2 protein (Bcl-2, anti-apoptotic protein) and decreasing Bcl-2-associated X protein (pro-apoptotic protein) expression (Chen *et al.*, 2010). On the other hand, two studies argue against this, as knockout of I-1 attenuates ISO-induced myocardial hypertrophy and arrhythmogenesis in mice (El-Armouche *et al.*, 2008), and over-expression of I-1 caused hyperphosphorylation of phospholamban and ryanodine receptors, resulting in generation of catecholamine-induced Ca^{2+} sparks (Wittkopper *et al.*, 2010).

Is there a relationship between adrenoceptors, MMPs and PPP?

From the data presented above, it is evident that there is an interrelationship between adrenoceptors and MMPs, and that PPP can control both. Therefore, it is reasonable to suggest that there may be crosstalk between the systems. Several pieces of circumstantial evidence support this paradigm. Firstly, okadaic acid abrogates the inhibitory effects of β_2-adrenoceptor stimulation on keratinocytes through recruitment of PP2A to the receptor, and increases the association between PP2A and ERK2 (Pullar *et al.*, 2003), both of which modulate the MMP system. Secondly, PKC, which is associated with adrenoceptor signalling, decreases MMP-2 activity through phosphorylation of serine/threonine residues, while dephosphorylation by alkaline phosphatase increases its activity (Sariahmetoglu *et al.*, 2007).

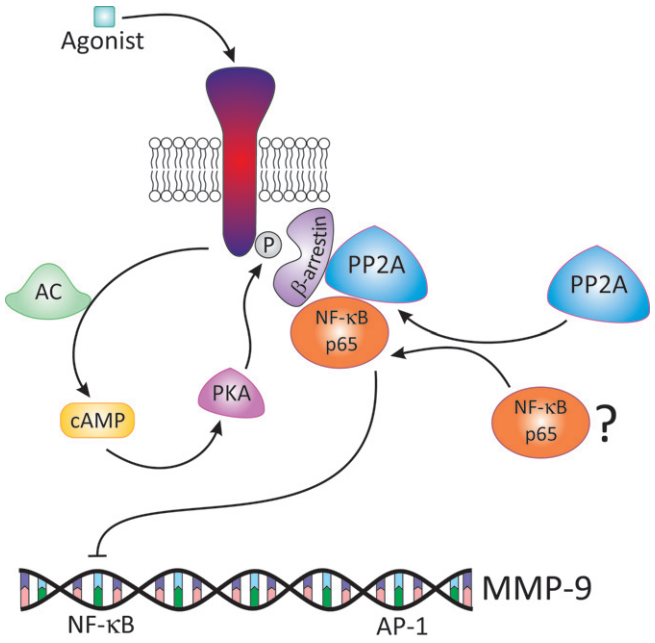

Figure 5

A model of the proposed mechanism by which β-adrenoceptor activation may down-regulate MMP-9 expression. Following binding of an agonist to the β-adrenoceptor, there is an increase in intracellular cAMP levels and phosphorylation of the receptor by PKA or GRK. As a result of this, β-arrestin is recruited to the receptor. Agonist stimulation also activates PP2A and initiates formation of a tri-molecular complex with β-arrestin and the p65 subunit of NF-κB. The trimolecular complex then interacts with the NF-κB binding motif on the MMP-9 promoter to inhibit transcription in a non-classical manner.

More convincingly, Rietz *et al*. recently demonstrated that the inhibitory effect of ISO on okadaic acid-mediated MMP-9 expression is blocked by siRNA knock-down of PP2A in NIH3T3 fibroblasts, indeed indicating communication between all three signalling components. Mechanistically, this involves β-arrestin-2 and a consensus sequence for NF-κB on the MMP-9 promoter, but not NF-κB itself (Rietz *et al*., 2012). Although the mechanism by which these components interact remains to be determined, we postulate that β-arrestin-2 forms a complex with PP2A and NF-κB upon β-adrenoceptor activation, which inhibits MMP-9 gene expression in a non-classical way (Figure 5). Consistent with this, β-arrestin can form a scaffolding complex resulting in activation of ERK1/2 (Luttrell *et al*., 1999; Defea *et al*., 2000), and associates with PP2A following exposure to insulin to disrupt G-protein-mediated MAPK signalling (Hupfeld *et al*., 2005). Finally, β-arrestin can inhibit NF-κB activity (Witherow *et al*., 2004), an important transcription factor implicated in transcriptional regulation of several MMPs and TIMPs (Yan and Boyd, 2006).

Conclusion and outlook

While these recent studies support crosstalk between adrenoceptors, MMPs and PPP, the implications of this paradigm, along with delineation of the signalling mechanisms, remain to be fully understood. Moreover, it will be particularly relevant to determine how this communication is altered in disease, since several diseases, including heart failure and cancer, are associated with alterations in adrenoceptor function, MMP expression and PPP activity. Indeed data from current and future studies may have broad implication for our understanding of the immunomodulatory effects of targeting the adrenergic system and may identify new targets for pharmacological manipulation of the MMP system. However, the communication between these three pivotal signalling elements does lead us to conclude exciting prospects in future investigation of disease remodelling.

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Conflict of interest

None.

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