

# INTERNATIONAL UNION OF BASIC AND CLINICAL PHARMACOLOGY REVIEW

## Significance of angiotensin 1–7 coupling with MAS1 receptor and other GPCRs to the renin-angiotensin system: IUPHAR Review 22

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**Received** 24 November 2016; **Revised** 31 January 2017; **Accepted** 6 February 2017

This paper, written by members of the International Union of Basic and Clinical Pharmacology Committee on Receptor Nomenclature and Drug Classification (NC-IUPHAR) subcommittees for the angiotensin receptors, confirms the existing nomenclature for these receptors and reviews our current understanding of their structure, pharmacology and functions, and their likely physiological roles in health and disease. More information on these receptor families can be found in the Concise Guide to PHARMACOLOGY (<http://onlinelibrary.wiley.com/doi/10.1111/bph.12445/abstract>); for classical angiotensin receptors, <http://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=6.>; and for MAS1, <http://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=150>.

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Angiotensins are a group of hormonal peptides and include angiotensin II and angiotensin 1–7 produced by the renin angiotensin system. The biology, pharmacology and biochemistry of the receptors for angiotensins were extensively reviewed recently. In the review, the receptor nomenclature committee was not emphatic on designating MAS1 as the angiotensin 1–7 receptor on the basis of lack of classical G protein signalling and desensitization in response to angiotensin 1–7, as well as a lack of consensus on confirmatory ligand pharmacological analyses. A review of recent publications (2013–2016) on the rapidly progressing research on angiotensin 1–7 revealed that MAS1 and two additional receptors can function as ‘angiotensin 1–7 receptors’, and this deserves further consideration. In this review we have summarized the information on angiotensin 1–7 receptors and their crosstalk with classical angiotensin II receptors in the context of the functions of the renin angiotensin system. It was concluded that the receptors for angiotensin II and angiotensin 1–7 make up a sophisticated cross-regulated signalling network that modulates the endogenous protective and pathogenic facets of the renin angiotensin system.

### Abbreviations

Ang, Angiotensin; Ang(1–7), angiotensin(1–7); AngII, octapeptide angiotensin (Asp<sup>1</sup>-Arg<sup>2</sup>-Val<sup>3</sup>-Tyr<sup>4</sup>-Ile<sup>5</sup>-His<sup>6</sup>-Pro<sup>7</sup>-Phe<sup>8</sup>); AngIII, angiotensin 2–8 (Asp<sup>1</sup>-Arg<sup>2</sup>-Val<sup>3</sup>-Tyr<sup>4</sup>-Ile<sup>5</sup>-His<sup>6</sup>-Pro<sup>7</sup>); Angiotensin A, (Ala<sup>1</sup>-Arg<sup>2</sup>-Val<sup>3</sup>-Tyr<sup>4</sup>-Ile<sup>5</sup>-His<sup>6</sup>-Pro<sup>7</sup>-Phe<sup>8</sup>); AngIV, angiotensin 3–8 (Val<sup>3</sup>-Tyr<sup>4</sup>-Ile<sup>5</sup>-His<sup>6</sup>-Pro<sup>7</sup>-Phe<sup>8</sup>); RAS, renin-angiotensin system

## Tables of Links

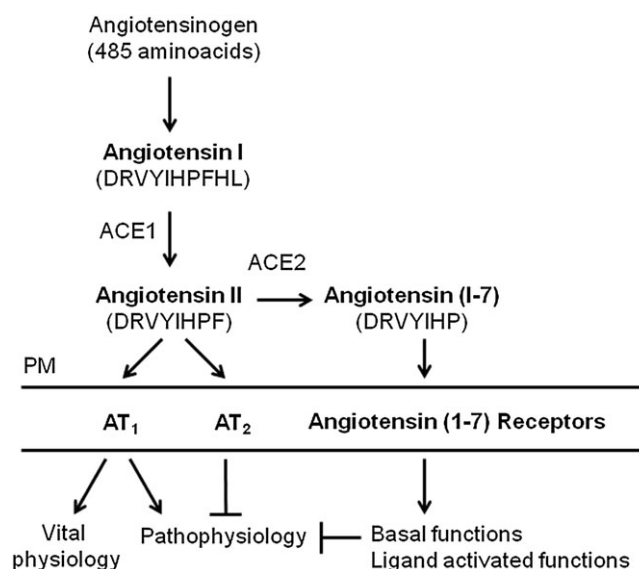
TARGETS	
<b>GPCRs<sup>a</sup></b>	<b>Enzymes<sup>b</sup></b>
AT <sub>1</sub> receptor	ACE1
AT <sub>2</sub> receptor	ACE2
B <sub>1</sub> receptor	p70S6K
B <sub>2</sub> receptor	Renin
MAS1 receptor	<b>Other protein targets<sup>c</sup></b>
MRGPRD (MrgD) receptor	TNF- $\alpha$

LIGANDS	
AngI	CGEN-857
Ang(1-9)	GDNF
AngII	ICAM-1
AngIII	IGF-1
Ang(1-7)	IL-1 $\beta$
Alamandine	MBP7
Arachidonic acid	MCP-1 (CCL2)
AR234960	Neuropeptide FF
AR244555	PD123319
AVE 0991	TGF $\beta$ 1
cAMP	VCAM-1
CGEN-856	

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Southan *et al.*, 2016), and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 (<sup>a,b,c</sup>Alexander *et al.*, 2015a,b,c).

## Introduction

The classical renin angiotensin system (RAS) regulates (i) the production of the hormone angiotensin II (AngII) from angiotensinogen, which involves renin and angiotensin converting enzyme 1 (ACE1), and (ii) the initiation of the homeostatic physiological response to AngII, predominantly through the AngII type 1 (AT<sub>1</sub>) receptor (Karnik *et al.*, 2015; Singh and Karnik, 2016). Both the production and cellular actions of this octapeptide hormone are critical body functions, because deletion of genes for either angiotensinogen or the AT<sub>1</sub> receptors increases mortality in mice (Tsuchida *et al.*, 1998; Doan *et al.*, 2001). In humans, clinical application of RAS blockers does not necessarily lower AngII, instead, an increase in tissue and circulating AngII levels is observed, suggesting that their therapeutic effects are probably due to metabolized AngII products. The presumptive therapeutic messenger of RAS is the heptapeptide metabolite, Ang(1-7). Ang(1-7) has been characterized as an antagonist of the pathophysiological effects of AngII (Ferrario *et al.*, 1991; Iyer *et al.*, 1998). Subsequently, the mono-carboxypeptidase ACE2 (Lemos *et al.*, 2002; Ren *et al.*, 2002) was shown to be the major enzyme producing Ang(1-7) *in vivo* from proteolysis of AngII. These discoveries led to the bifurcation of RAS with the addition of a new ACE2 arm (Figure 1), which produces Ang(1-7). ACE2 has been shown to cleave multiple peptide substrates and play a role in amino acid uptake in the gut. Moreover, ACE2-knockout mice have been extensively used to elucidate the *in vivo* functions of Ang(1-7). Recent studies have demonstrated that increasing ACE2 in tissues up-regulates levels of Ang(1-7), which are directly associated with a reduction in the progression of neuronal, renal and cardiovascular diseases including pulmonary arterial hypertension (PAH). Thus, the ACE2/Ang(1-7) axis may have evolved to counterbalance the pathophysiological effects of overactivation of the classical ACE1/AngII axis *in vivo*



**Figure 1**

The RAS including the novel ACE2/Ang(1-7) axis.

(Bradford *et al.*, 2010; Ahmad *et al.*, 2011; de Kloet *et al.*, 2013; Jiang *et al.*, 2014a,b). Characterization of receptor components of the ACE2/Ang(1-7) axis, however, is unfinished and currently in progress.

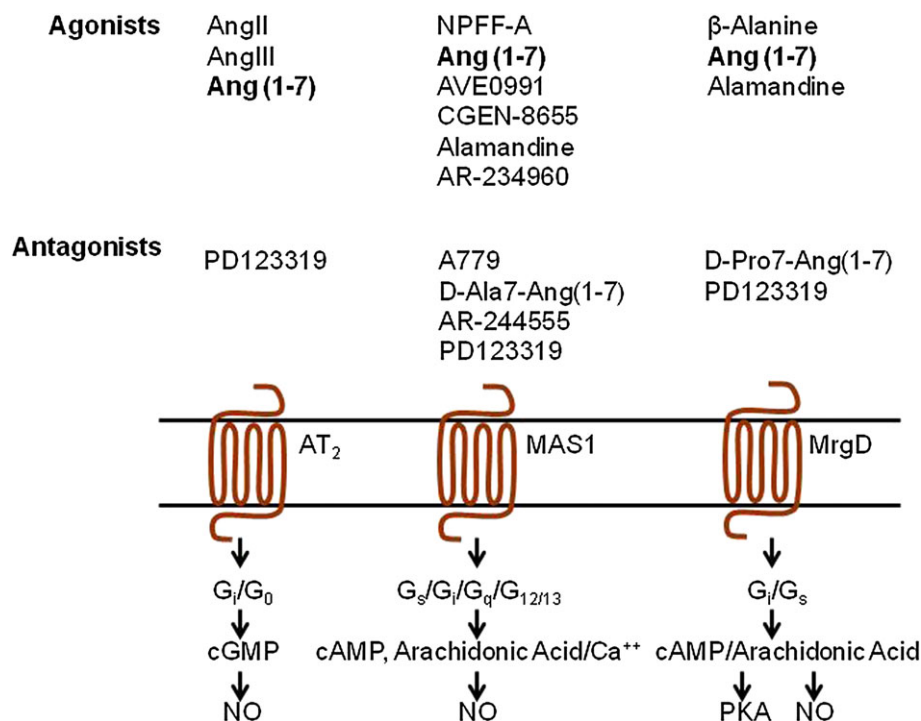
The finding that the GPCR, MAS (also called MAS1) mediates some of the actions of Ang(1-7) was a breakthrough in unravelling the body's response mechanism to Ang(1-7) (Santos *et al.*, 2003). Initially MAS1 was discovered as a growth promoting GPCR (Young *et al.*, 1986; Janssen *et al.*, 1988; Dean and Boynton, 1990; van't Veer *et al.*, 1993) and was erroneously proposed as a receptor for AngII and AngIII;

but this assignment was later withdrawn (Jackson *et al.*, 1988; Jackson and Hanley, 1989; McGillis *et al.*, 1989; Sasamura *et al.*, 1992). This time around, the investigations showed that Ang(1–7) elicited arachidonic acid release in MAS1-transfected cells. *Mas1* gene deleted mice are normal, but the binding of Ang(1–7) in their kidney slices is abolished, Ang(1–7)-induced relaxation response in the aorta is lost and the antidiuretic action of Ang(1–7) after an acute water load is abolished (Santos *et al.*, 2003). *Mas1* gene-null mouse tissues and organs respond poorly to Ang(1–7) (Walther *et al.*, 1998; Fraga-Silva *et al.*, 2008; Santos *et al.*, 2008; Mario *et al.*, 2012). However, independent laboratories have reported a lack of MAS1 receptor signalling in response to Ang(1–7) and a lack of evidence for direct Ang(1–7) binding to the MAS1 receptor (Bikkavilli *et al.*, 2006; Shemesh *et al.*, 2008; Zhang *et al.*, 2011; Tirupula *et al.*, 2014a). There is evidence in the literature for additional receptors mediating the effects of Ang(1–7) (Figure 2). For example, the AT<sub>2</sub> receptor was shown to bind Ang(1–7) (De Souza *et al.*, 2004; Walters *et al.*, 2005) and Ang(1–7) signals through AT<sub>2</sub> receptors in some studies (Jones *et al.*, 2011; Sipahi *et al.*, 2011; Ohshima *et al.*, 2014), although this is not replicated in all settings (Lautner *et al.*, 2013; Mendonca *et al.*, 2014; Tetzner *et al.*, 2016). De-orphanization efforts on the MAS1-related GPCR (MRGPR) family have uncovered potential new receptors for consideration in the ACE2/Ang(1–7) pathway (Gembarde *et al.*, 2008; Solinski *et al.*, 2014). Functional studies indicate that the MRGPRD (MrgD) receptor mediates acute and persistent responses to Ang(1–7) and its metabolite alamandine in target tissues akin to those produced by MAS1 receptors (Habiyakare *et al.*, 2014; Wilson *et al.*, 2015; Tetzner *et al.*, 2016).

The confusing pharmacology and transduction modalities for MAS1, the awareness of ligand-promiscuity among MRGPRs and evidence of MRGPRs responding to other angiotensin metabolites (Bader *et al.*, 2014) have delayed a conclusive designation of MAS1 receptors and other candidate MRGPR receptors as the Ang(1–7) receptor by the IUPHAR/BPS subcommittee (Karnik *et al.*, 2014; 2015; Singh and Karnik, 2016). In addition, the classification in *Pharmacological Reviews* (Davenport *et al.*, 2013; Solinski *et al.*, 2014) and the IUPHAR/BPS guide to pharmacology Database (<http://www.guidetopharmacology.org/>) have included MAS1 and MRGPRD (MrgD) in the list of ‘orphan’ GPCRs for which pairing with the endogenous ligand(s) is inconclusive. The aim of this review is to provide the present status of the field of candidate Ang(1–7) receptors, with focus on recent important findings and particularly the challenges for future research.

## The progress in coupling Ang(1–7) functions with MAS1 receptors

MAS1 was originally identified as the oncogenic GPCR in skin cancer cell lines and is currently catalogued under a group of ~40 orphan MRGPRs by the HUGO gene nomenclature committee and the IUPHAR/BP database (Monnot *et al.*, 1991; Dong *et al.*, 2001; Gembarde *et al.*, 2008; Tirupula *et al.*, 2014b). Tissue distribution of the MAS1 receptor is ubiquitous; it is abundantly expressed in the brain and testes whereas its expression is low in the heart, kidneys, lungs, vasculature, adipose tissue and skeletal muscle. The MAS1 receptor may regulate many vital body functions and its



**Figure 2**

A current view of the three receptors proposed to signal in response to Ang(1–7). MrgD receptor is also known as MRGPRD receptor.

dysfunction may lead to various pathologies. There is emerging evidence suggesting an important role for Ang(1–7)'s interaction with MAS1 receptors in the function of the brain as well as that of multiple organs including the kidney, heart, vasculature, skeletal muscle, retina and the liver. MAS1 is a constitutively active GPCR; hence, changes in MAS1 expression levels could affect signalling pathways and thus may function without requiring a ligand. However, the MAS1 receptor is reported to bind various endogenous ligands including Ang(1–7), alamandine and neuropeptide FF (NPFF) and dimerize with other GPCRs (Figure 2). However, the physiological significance of these diverse mechanisms remains unclear. In this section, we summarize findings published in past 3 years ending August 2016 on the physiology and pathology involving MAS1 receptors linked to the ACE2/Ang(1–7) pathway (Bader, 2013; Solinski *et al.*, 2014; Karnik *et al.*, 2015). In reviewing recent functional studies on MAS1 receptors, we have tried to limit the inclusion of observations supported by a single publication. We have strived, in vain, to find unequivocal pharmacological evidence for a formal adoption of the MAS1 receptor as the Ang(1–7) receptor.

The promise of novel therapeutic approaches targeting the ACE2/Ang(1–7) axis appears to be the major impetus for the continuing interest in MAS1 functions in the brain for treatment of ischaemic stroke and cerebrovascular diseases (see Figure 1). Enhancing systemic ACE2/Ang(1–7) axis is thought to attenuate the development of hypertension and the pathological progress of atherosclerosis and prevent thrombotic events, which may contribute to a reduced risk of ischaemic stroke, a reduction in cerebral infarct size and amelioration of neurological deficits; these are thought to be mediated through its antioxidative and anti-inflammatory effects. Excellent reviews that highlight these topics are available (Bader, 2013; Jiang *et al.*, 2013; Montezano *et al.*, 2015; Silva and Pinheiro, 2015; Bennion *et al.*, 2015a,b; Colafella *et al.*, 2016; Machado-Silva *et al.*, 2016; Simoes and Teixeira, 2016)

The neuroprotective effects mediated by enhancing ACE2 or Ang(1–7) in ischaemic and haemorrhagic stroke have spurred interest in neuroprotective mechanisms of MAS1 in the clinical setting. The expression of ACE2 and MAS1 receptors are up-regulated after acute cerebral ischaemic stroke in rats (Lu *et al.*, 2013). Also a deficiency in MAS1 receptors has been found to exacerbate cerebral and systemic inflammation in mice (Oliveira-Lima *et al.*, 2015). In stroke-prone spontaneously hypertensive rats, a haemorrhagic stroke model, centrally administered Ang(1–7) increased the survival of the rats, suggesting that the MAS1 receptor is a potential therapeutic target in haemorrhagic stroke (Sumners *et al.*, 2013; Regenhardt *et al.*, 2013a,b). When Ang(1–7) is injected into regions of the hypothalamus that control cardiovascular function, the BP is reduced. However, Ang(1–7) injection into the rostral ventrolateral medulla increases BP. It has been suggested that Ang(1–7) has a critical role in the control of baroreflex sensitivity and hypothalamic noradrenergic neurotransmission during the development of hypertension (Gironacci *et al.*, 2014; Bennion *et al.*, 2015a,b). Cerebral damage is enhanced in mice overexpressing human renin and angiotensinogen and experimental ischaemic stroke induced in brain slices from these mice *in vitro* by oxygen

and glucose deprivation caused tissue swelling, which was associated with the production of ROS and cell death. When these mice were treated with Ang(1–7) the damaging effects were reduced and treatment with the MAS1 antagonist A-779 eliminated this protective effect of Ang(1–7) (Regenhardt *et al.*, 2013c; Zheng *et al.*, 2014a). Infusion of Ang(1–7) induces cerebral ischaemic tolerance by promoting brain angiogenesis in a MAS1/eNOS-dependent pathway which is reversed by the specific MAS1 antagonist A-779 (Jiang *et al.*, 2014a,b; Zheng *et al.*, 2014b). The mortality rate due to rupture of intracranial aneurysms in mice is reduced by intracranial injection of Ang(1–7) (Pena Silva *et al.*, 2014). In diabetic rats Ang(1–7) treatment up-regulates the expressions of GFAP and GDNF promoting neuron survival in the hippocampus, and this effect was blocked by treatment with A-779 (Zhang *et al.*, 2015a). In chronic neurogenic hypertension microglial activation and the production of pro-inflammatory cytokines, IL-1 $\beta$  and TNF $\alpha$ , and the anti-inflammatory cytokine IL-10 can be abolished by Ang(1–7). In contrast the MAS1 antagonist A-779 enhances inflammation and microglial activation suggesting that 'neurogenic' hypertension may be targeted by MAS1 (Liu *et al.*, 2015).

The overexpression of ACE2 in the brain was found to activate central MAS1-induced spontaneous postsynaptic inhibitory currents, indicative of presynaptic GABA release onto pyramidal neurons, which reduced anxiety-like behaviour in mice; the MAS1 antagonist A-779 eliminated this effect. Furthermore, centrally administering A-779 abolished the anxiolytic phenotype in ACE2-null mice (Oscar *et al.*, 2014; Fontes *et al.*, 2015; Wang *et al.*, 2016). Indeed, activation of the brain Ang(1–7)/MAS1 axis in hypertensive transgenic (mRen2)27 rats lowered BP and attenuated cardiac remodelling by improving the autonomic balance (Kangussu *et al.*, 2015). In female rats, central Ang(1–7) protects against aldosterone/NaCl-induced hypertension, by decreasing heart rate and renal sympathetic nerve activity (Xue *et al.*, 2013). In Sprague–Dawley rats, Ang(1–7) injected into the nucleus tractus solitarius (NTS) decreases BP, heart rate and renal sympathetic nerve activity through the generation of NO (Wu *et al.*, 2015).

Ang(1–7) mediated MAS1 activation has been evaluated as an antinociceptive intervention. Treatment with Ang(1–7) significantly attenuated bone pain suggesting it could be used in the relief of excruciating pain in advanced stage cancer patients (Forte *et al.*, 2016). Intraplantar peripheral hyperalgesia induced by PGE<sub>2</sub> was reversed by a low-dose of Ang(1–7) (Costa *et al.*, 2014; Nemoto *et al.*, 2014; Castor *et al.*, 2015).

In animal studies and in small human subgroups, treatments selectively targeting different components of the RAS are changing the perspectives for the prevention of the pathophysiology of heart failure and coronary atherothrombosis. In renal arteries from diabetic patients, *ex vivo* Ang(1–7) treatment reduced oxidative stress (Zhang *et al.*, 2015b). The central ACE2/Ang(1–7) axis is a key player in the regulation of sympathetic outflow in chronic heart failure (Zucker *et al.*, 2014), the risk of adverse cardiovascular events in response to acute emotional stress (Martins Lima *et al.*, 2013; Xing *et al.*, 2014), and also cellular hypertrophy and myofibroblast transformation (Alzayadneh and Chappell, 2014). Vascular sympathetic modulation mediated through

the ACE2/Ang(1-) axis is well-documented (Rabello Casali *et al.*, 2016). These studies indicate that Ang(1-7) is involved in producing NO through MAS1, which contributes to the variations in heart rate in the autonomic modulation of arterial pressure and cardiovascular regulation (Abwainy *et al.*, 2015; da Silva *et al.*, 2015; Zhang *et al.*, 2015c). An increased expression of the ACE2/Ang(1-7) axis is observed in hyperthyroidism-induced cardiac hypertrophy (Diniz *et al.*, 2015) and in a pressure overloaded rat model (Liang *et al.*, 2015a,b). In an acute atrial tachycardia canine model, Ang(1-7)/MAS1/PI3K/Akt/NO/atrial natriuretic peptide prevented acute electrical remodelling (Zhao *et al.*, 2014). Cardioprotection by Ang(1-7) against AngII-induced cardiac remodelling through inhibition of cardiomyocyte autophagy is also reported (Lin *et al.*, 2015). Pharmacological blockade or genetic deletion of MAS1 attenuates physiological cardiac hypertrophy that can occur during pregnancy (Carmos-Silva *et al.*, 2016). Treatment with Ang(1-7) reduces inflammation in carotid atherosclerotic plaques (Fraga-Silva *et al.*, 2014). Long-term administration of Ang(1-7) prevents inflammation and pathological remodelling of the heart and lung in a mouse model of type 2 diabetes (Hao *et al.*, 2015; Papinska *et al.*, 2016). Ang(1-7) reverses hyperglycaemia and its consequences in an animal model of type 2 diabetes (Santos *et al.*, 2013). Ang(1-7) regulates insulin secretion through a MAS-dependent cAMP signalling pathway (Sahr *et al.*, 2016).

Analysis of muscular expression of various components of the RAS in patients with achalasia has shown the existence of local RAS in human oesophageal and skeletal muscle. In the epithelium of patients with healthy and acid reflux-exposed human oesophageal mucosae, the expression of RAS components, particularly ACE2, ACE, AT<sub>1</sub> and AT<sub>2</sub> receptors and MAS1, was reported to vary (Bjorkman *et al.*, 2012). In patients with achalasia, a shift in receptor physiology from AT<sub>1</sub> receptor to MAS1 receptor is reported (Casselbrant *et al.*, 2014). The healing of pre-existing gastric ulcers is associated with an up-regulation of Ang(1-7), MAS1, NO, prostaglandins and pro-inflammatory cytokines and a decreased expression of AT<sub>1</sub> receptors has been observed during the local vascular and metabolic effects associated with gastric ulcer healing (Pawlik *et al.*, 2016). The gastric mucosal protection mechanism involves a similar scenario (Brzozowski, 2014; Lu *et al.*, 2014). After the induction of experimental colitis, the colonic expression of ACE2, Ang1-7 and MAS1 was enhanced and daily Ang(1-7) treatment significantly reduced colitis, whereas administration of A-779 significantly increased colitis (Khajah *et al.*, 2016).

In skeletal muscle, RAS activation induces insulin resistance and impairs glucose uptake, which is counterbalanced by actions of the ACE2/ANG(1-7)/MAS1 axis (Henriksen and Prasannarong, 2012). Ang(1-7) affects muscle microvasculature and enhances insulin's metabolic action via the MAS1 receptor (Fu *et al.*, 2014). Chronic oral administration of Ang(1-7) has been shown to improve skeletal muscle insulin sensitivity, and autonomic and locomotor phenotypes in muscular dystrophy (Echeverria-Rodriguez *et al.*, 2013; Gomes-Santos *et al.*, 2014; Sabharwal *et al.*, 2014; Morales *et al.*, 2014a,b). Endotoxin-induced skeletal muscle wasting can be prevented by Ang(1-7) through a p38 MAPK-dependent mechanism. Ang(1-7) prevents the decrease in the diameter of myofibres and myotubes, and decrease in

muscle strength induced by LPS. These effects were reversed by the MAS1 receptor antagonist A-779 (Cabello-Verrugio *et al.*, 2015; Morales *et al.*, 2015). Ang(1-7) reduces myonuclear apoptosis during recovery from AngII-induced skeletal muscle atrophy in mice (Meneses *et al.*, 2014), and attenuates signalling involving TGF- $\beta$  both *in vitro* and *in vivo* (Morales *et al.*, 2014a,b). The oral administration of Ang(1-7) in a mouse model of Duchenne muscular dystrophy (DMD) normalized skeletal muscle architecture, decreased local fibrosis and improved muscle functions. These effects are mediated through inhibition of TGF- $\beta$  Smad signalling (Acuna *et al.*, 2013). Ang(1-7) could contribute to weight gain in this model (Schuchard *et al.*, 2015).

Recent reviews on kidney diseases discuss new research elucidating the role of ACE1 and ACE2 in haemodialysis patients, which may aid the development of targeted therapies that slow the progression of chronic kidney and cardiovascular diseases (Carey, 2015; Malik and Raizada, 2015; Oparil and Schmieder, 2015; Te Riet *et al.*, 2015). The anti-inflammatory effects of Ang(1-7) could ameliorate high fat diet-induced renal injury. The administration of Ang(1-7) significantly improved the inflammatory status, down regulated LDLr, SREBP2 and SCAP, and then, decreased the deposits of lipid in the kidney and improved renal injury (Olivon *et al.*, 2015; Zheng *et al.*, 2015b). An up-regulation in the expression of ACE2/AT<sub>2</sub> receptors and MAS1 receptors in diabetic mice is associated with the renoprotective effects of candesartan in db/db mice suggesting that the ACE-2/Ang(1-7) axis can be a therapeutic target for diabetes-induced hypertension and renal damage (Callera *et al.*, 2016; Padda *et al.*, 2016). The endothelial progenitors may be dysfunctional in diabetic mice. In patients with diabetes, Ang(1-7) overexpression by lentiviral delivery restored both the *in vitro* vaso-reparative functions and the *in vivo* homing efficiency of the peripheral blood CD34+ cells to areas of ischaemia compared with those of nondiabetic controls. In a cohort of patients who had higher expression of ACE2/MAS1 receptors than diabetic patients, the development of CD34+ cell dysfunction was reduced. Thus, activating the ACE2/Ang(1-7)/MAS1 receptor axis could overcome endothelial dysfunction and enhance the reparative function of endothelial progenitors (Jarajapu *et al.*, 2012; Singh *et al.*, 2015).

The expression of MAS1 receptors and other components of the renin-angiotensin system were shown in the human eye, suggesting that MAS1 receptors may have a role in physiological and pathological processes in the eye and in the retina (Vaajanen *et al.*, 2015). The expression and cellular localization of MAS1 receptors in the adult and developing mouse retina is also documented (Prasad *et al.*, 2014) the activation of intrinsic ACE2 has been shown to have anti-glaucomatous effects (Foureaux *et al.*, 2013). Several independent reports suggest that deletion of ACE2 leads to vascular diseases (Thomas *et al.*, 2010; Thatcher *et al.*, 2011; Sahara *et al.*, 2013; Rabelo *et al.*, 2016). Furthermore, the MAS axis may be an interesting therapeutic target for multiple sclerosis and atherosclerosis (Hammer *et al.*, 2016) and hypothermia (Souza *et al.*, 2014). Activation of MAS could be an important target for the chronic hepatic and metabolic alterations during atherosclerosis (Silva *et al.*, 2013). An impairment of the Ang(1-7)/Mas receptor pathway may lead to a worsening

of the pathophysiological changes in asthma (Magalhaes *et al.*, 2016).

## Experimental model studies

Central Ang(1–7) treatment of hypertensive (mRen2)27 transgenic rats attenuates the anxiety and depression-like behaviour in these animals (Almeida-Santos *et al.*, 2016; Wang *et al.*, 2016). In a chronic constriction injury rat model, intrathecal administration of Ang(1–7) relieves, whereas A-779 aggravates injury-induced neuropathic pain (Zhao *et al.*, 2015). The MAS1-null mice may serve as an animal model to study post-traumatic stress disorder. Ang(1–7)/MAS1 axis deficits may accelerate memory extinction in the MAS1-null mice (Lazaroni *et al.*, 2015). In a mouse model of Alzheimer's disease, i.c.v. infusion of Ang(1–7) ameliorated the cognitive impairments and memory dysfunction (Uekawa *et al.*, 2016). In models of Alzheimer's and Parkinson's diseases, Ang(1–7) activity correlates with tau hyperphosphorylation (Wright *et al.*, 2013; Jiang *et al.*, 2015). The Ang(1–7)/MAS1 axis affects the proliferation of the population of doublecortin positive cells within the dentate gyrus and the piriform cortex. A deficiency of MAS1 in the null mice increases the number of DCX-positive young neurons, suggesting that blockade of MAS1 might be beneficial in stimulating neurogenesis in adults (Freund *et al.*, 2013).

Ang(1–7) counteracted the inflammation involved in vascular remodelling and haemorrhagic stroke in cerebral micro vessels by affecting the NF $\kappa$ B-mediated expression of TNF- $\alpha$ , MCP-1 and IL-8 (Bihl *et al.*, 2015). The cardiomyopathy and diastolic dysfunction in diabetic (db/db) mice is ameliorated by Ang(1–7) (Mori *et al.*, 2014). Long-term administration of Ang(1–7) prevents heart and lung dysfunction in db/db mice (Papinska *et al.*, 2016). In Ren-2 transgenic rats exposed to chronic hypoxia, intrapulmonary activation of the ACE2/Ang(1–7)/MAS1 axis attenuates pulmonary hypertension (Hampl *et al.*, 2014). In an acute lung injury model, Ang(1–7) attenuates lung fibrosis (Chen *et al.*, 2013). The ACE2/Ang(1–7)/MAS1 axis inhibits the MAPK/NF- $\kappa$ B pathway to protect against lung fibrosis (Meng *et al.*, 2013). In a rat model of neonatal hyperoxia-induced lung injury, MAS1 and AT<sub>2</sub> receptor agonists attenuate cardiopulmonary disease (Wagenaar *et al.*, 2013). In a model of chronic allergic lung inflammation, Ang(1–7) attenuates lung inflammation, airway remodelling and hyperresponsiveness (Magalhaes *et al.*, 2015; Murugan *et al.*, 2015). Deletion of the MAS1 gene significantly increased intimal proliferation, increasing the aortic intima : media ratio, which promotes atherosclerosis (Alsaadon *et al.*, 2015).

The role of RAS has been examined in models of liver disease model, so as to evaluate it as a target for the treatment and prevention of liver dysfunction (Moreira de Macedo *et al.*, 2014). The ACE2/Ang(1–7)/MAS1 axis activates Akt signalling to ameliorate hepatic steatosis. Deletion of the ACE2 gene aggravates liver steatosis and insulin resistance, increases the expression of hepatic lipogenic genes and decreases the expression of fatty acid oxidation genes in mice (Cao *et al.*, 2016). In ACE2 knockout mice, Ang(1–7) alleviates insulin resistance and oxidative stress (Cao *et al.*, 2014).

In a model of acute reflux oesophagitis, Ang(1–7) exerts a protective effect on the oesophagus through the involvement of NO, sensory nerve hypoxia-inducible factor-1 $\alpha$  and pro-inflammatory cytokines IL-1 $\beta$  and TNF- $\alpha$  (Pawlik *et al.*, 2015). In an acetic acid-induced gastric ulcer model, Ang(1–7) accelerates the healing of pre-existing gastric ulcers by increasing the macro- and microcirculation, which increases gastric tissue oxygenation. Blockade of MAS1 receptors by A-779 abolishes this healing effect of Ang(1–7) mediated by PGs and NO, and increases the expression of the antioxidizing enzyme SOD 2 and cytokines IL-1 $\beta$  and TNF- $\alpha$  (Pawlik *et al.*, 2016). Ang(1–7) attenuates disuse atrophy in skeletal muscle. The classical RAS is activated in the immobilized hind limb of C57BL6 mice and Ang(1–7) treatment increased anti-atrophic signalling by IGF-1/IGFR-1/Akt/p70S6K and FoxO3. These effects of Ang(1–7) were not observed in MAS1 knockout mice (Morales *et al.*, 2016). Ang(1–7) restored muscle strength in dystrophic muscles through the inhibition of TGF- $\beta$  signalling in a mouse model of Duchenne muscular dystrophy (Morales *et al.*, 2016).

Kidney injury caused by unilateral ureteral obstruction (UUO) in a Sprague–Dawley rat model of nephropathy was attenuated by Ang(1–7). Renal tubulointerstitial apoptosis, expression of profibrotic proteins, pro-apoptotic proteins and (TGF)- $\beta$ 1/Smad signalling, were reduced by Ang(1–7) treatment. Treatment with A-779 and MAS1 receptor siRNA enhanced Ang II-induced apoptosis and fibrosis (Kim *et al.*, 2015). Ang(1–7) normalizes renal ACE2 and MAS1 receptor expression in Type 1 diabetic Akita mice (Shi *et al.*, 2014). Ang(1–7) attenuates the progression of streptozotocin-induced renal injury, and this is accompanied by a decreased expression of collagen IV, TGF- $\beta$ 1, VEGF, NOX4, p47phox, PKC $\alpha$  and PKC $\beta$ 1, and the phosphorylation of Smad3 (Zhang *et al.*, 2014). Ang(1–7) modulates renal vascular resistance through inhibition of p38 MAPK in apolipoprotein E-deficient mice (Potthoff *et al.*, 2013).

## Confusing evidence

The results of the studies summarized above support the identification of MAS1 as the receptor mediating the *in vivo* effects of Ang(1–7). This designation is primarily based on the loss of Ang(1–7)-mediated functional responses in MAS1-null animal models and the antagonism of MAS1-regulated physiology by the specific antagonist, A-779 (Santos *et al.*, 1994; 2003). In some studies, surrogate MAS1-specific agonists AVE0991 (Wiemer *et al.*, 2002) and CGEN-856S (Shemesh *et al.*, 2008) and another antagonist and D-Pro7-Ang(1–7) have been used, but not regularly. However, a direct assessment of the functional effects of Ang(1–7) in different vascular tissues in humans, rats and various disease models has raised some concerns regarding the pairing of Ang(1–7) with the MAS1 receptor. In a recent study, the therapeutic potential of Ang(1–7) for the treatment of PAH was found to be limited as opposed to the huge benefits reported in other models of cardiovascular diseases. The effectiveness of Ang(1–7) and a stable, cyclic analogue, cAng(1–7), was found to be limited in a rat experimental monocrotaline (MCT) model of PAH. Treatment reduced right ventricular

systolic pressure, but this reduction was <50% and not significant (Breitling *et al.*, 2015).

In human mammary artery (HMA) samples obtained from patients undergoing coronary revascularization surgery, MAS1 expression was confirmed in endothelium. Also, acute Ang(1–7) treatment attenuates the AngII-induced constriction of HMA explants. Unexpectedly, pre-incubation with the specific MAS1 antagonist A-779 did not abolish this effect of AngII. Furthermore, the Ang(1–7) effect observed in HMA was not altered by the AT<sub>2</sub> receptor antagonist PD123177, indicating a pharmacologically distinct receptor in HMA (Mendonca *et al.*, 2014).

In the cirrhotic rat liver Ang(1–7) induces a vasodilator response and the activation of endothelial NOS (eNOS) by phosphorylation. This effect of Ang(1–7) was not blocked by MAS1-selective antagonist A-779 or by blockade of AT<sub>1</sub>, AT<sub>2</sub> and bradykinin B<sub>2</sub> receptors. Unexpectedly, the observed Ang(1–7) effects were not reproduced by MAS1-selective agonist AVE0991. However, the D-Pro<sup>7</sup>-Ang(1–7) analogue antagonized all the functional effects of Ang(1–7). These findings suggest that the Ang(1–7)-induced beneficial effects in cirrhosis are mediated by receptors that are pharmacologically distinct from the MAS1 receptor (Herath *et al.*, 2012). In the splanchic vessels of patients and rat cirrhosis model similar observations were reported (Grace *et al.*, 2013). Pharmacological evidence for the existence of a distinct Ang(1–7) receptor was obtained in the aorta from Sprague–Dawley rats, also the Ang(1–7)-induced vasodilator effect was not blocked by A-779, but was specifically abolished by D-Pro<sup>7</sup>-Ang(1–7) (Silva *et al.*, 2007). Altogether, these findings indicate the existence of an Ang(1–7) receptor that is not sensitive to A-779.

## MAS1-related GPCR MRGPRD (MrgD) coupling with Ang(1–7)/alamandine

Characterization of the MAS1-related GPCR (MRGPR) subfamily may help better understand the confusion as regards Ang(1–7) pairing with the MAS1 receptor. More than 40 members of this receptor family have been identified in multiple species. They are expressed in sensory neurons, and thought to be involved in the perception of itch, pain and pathological pain (Solinski *et al.*, 2014; Tiwari *et al.*, 2016). Most MRGPR are orphan receptors. However, de-orphanization efforts have led to new receptors pairing with the ACE2/Ang(1–7) pathway (Figure 2). For instance, while screening an assortment of MRGPRs for arachidonic acid release in response to angiotensin metabolites, Gemhardt *et al.* discovered that in addition to the MAS1 receptor, Ang IV [Ang(3–8)] stimulated AA release in MRGPRD (MrgD) and MRGPRX1 (Mrg1) transfected cells. AngIII activated MAS1 and MRGPRX2 receptors. This initial study lead to further characterization of the MRGPR family of GPCRs as potential targets of Ang(1–7) and related peptides (Gemhardt *et al.*, 2008).

The MRGPRD receptor may mediate NO dependent vasodilator effects of a relatively new heptapeptide, alamandine, which structurally differs from Ang(1–7) with an Ala replacing the N-terminal Asp and is thought to be produced *in vivo* from Ang A by the action of ACE2 (Lautner *et al.*, 2013). Ang A

has Ala<sup>1</sup> instead of Asp<sup>1</sup> in AngII. It was first identified in human plasma. Alamandine was identified in rats, mice and humans, where it is thought to produce the same effects as Ang(1–7), but by interacting with the MRGPRD receptor (Mendoza-Torres *et al.*, 2015). The alamandine-induced effects have been shown to be abolished by D-Pro-Ang(1–7), and the AT<sub>2</sub> receptor antagonist PD123319. The binding of alamandine to the MRGPRD receptor is blocked by the MRGPRD agonist β-alanine, D-Pro<sup>7</sup>-Ang(1–7) and PD123319, but not by the MAS1 antagonist A-779. Direct radioligand binding and rigorous pharmacological analysis data were provided to support the claim that MRGPRD is the alamandine receptor (Habiyakare *et al.*, 2014; Wilson *et al.*, 2015). MRGPRD or MRGPRD-like receptors may explain the findings that in certain circumstances Ang(1–7) effects are not blocked by A-779 (Etelvino *et al.*, 2014; Solinski *et al.*, 2014; Villela *et al.*, 2014a,b).

A recent study independently claimed that MRGPRD is the receptor for Ang(1–7) in addition to alamandine and the AT<sub>2</sub> receptor antagonist, PD123319 (Lautner *et al.*, 2013; Tetzner *et al.*, 2016). Using the cAMP readout assay (instead of arachidonic acid release assay), the authors showed functional activation of MAS1 receptors by Ang(1–7) in transfected HEK293 cells, contradicting data from previous independent studies (Bikkavilli *et al.*, 2006; Shemesh *et al.*, 2008; Zhang *et al.*, 2011; Tirupula *et al.*, 2014a). Furthermore, the authors presented data showing that MRGPRD functions as a receptor for Ang(1–7). In their analysis, Ang(1–7) failed to increase cAMP levels in primary mesangial cells obtained from MAS1 and MRGPRD double knockout mice. The haemodynamic response to Ang(1–7) was also impaired in MRGPRD-null mice. In this study, the Ang(1–7) stimulated arachidonic acid release was modest compared to the cAMP response in MRGPRD-transfected heterologous cells. Furthermore, the authors demonstrated polypharmacology of PD123319, which blocks the signalling by MAS1 and MRGPRD receptors as well as from the AT<sub>2</sub> receptor.

## AT<sub>2</sub> receptor coupling with Ang(1–7)

Reports from multiple laboratories have highlighted an Ang(1–7)/AT<sub>2</sub> receptor axis as a new protective arm of the RAS (Gaspari *et al.*, 2012). AT<sub>2</sub> receptor-dependent protective effects of Ang(1–7) against aneurysmal subarachnoid haemorrhage development in mice is reported. The protective effect of Ang(1–7) was not observed in AT<sub>2</sub> receptor knockout mice. Furthermore, the protective effects of Ang(1–7) were blocked by the AT<sub>2</sub> receptor antagonist PD123319, but not by the MAS1 receptor antagonist A-779. These findings indicate that protective effect of Ang(1–7) against aneurysmal rupture is mediated by AT<sub>2</sub> receptors (Shimada *et al.*, 2015). AT<sub>2</sub> receptor expression is up-regulated in many disease states suggesting a role for this receptor in disease (Gaspari *et al.*, 2012). In the apolipoprotein E<sup>-/-</sup> mouse model of atherosclerosis, Ang(1–7) can act via the AT<sub>2</sub> receptors to exert vasoprotective and atheroprotective effects and these effects of Ang(1–7) can be abolished by either the AT<sub>2</sub> receptor antagonist PD123319 or A-779 (Pernomian *et al.*, 2015). AT<sub>2</sub> receptor stimulation by Ang(1–7) has been shown to lower BP in adult normotensive rats (Walters *et al.*, 2005).

Furthermore, the effects of Ang(1–7) on vascular remodelling and its ability to inhibit neointimal growth are attenuated in AT<sub>2</sub> receptor knockout mice compared with wild-type mice. Surprisingly, Ang(1–7)-dependent attenuation of neointimal formation after vessel injury in the *MAS1*-null mice requires an increased expression of AT<sub>2</sub> receptors (Ohshima *et al.*, 2014). In a mouse model of UUO, the administration of Ang(1–7) caused injury in obstructed kidneys compared with controls and increased macrophage infiltration. In mice with *MAS1* gene deletion, the delivery of exogenous Ang(1–7) worsens kidney damage. Surprisingly, an infusion of Ang(1–7), but not A-779 further increased apoptosis and macrophage influx in obstructed kidneys from *MAS1*-null mice compared with untreated mice (Zimmerman *et al.*, 2015). Could these effects be due to an unknown receptor or are mediated by the AT<sub>2</sub> receptor? Taken together, these findings suggest that the AT<sub>2</sub> receptor is an important mediator of effects attributed to the ACE2/Ang(1–7) axis.

## Pharmacology

The suggestion that Ang(1–7) is the physiological agonist for the MAS1 receptor is based on the loss of physiological effects of Ang(1–7) and reduced [<sup>125</sup>I]-Ang(1–7) binding to kidney sections in *MAS1*-null mice (Santos *et al.*, 2003; Pinheiro *et al.*, 2004a,b). Generally, specificity and a dissociation constant for the ligand or receptor density are not measured in these types of experiments. Despite impressive advances made through functional characterization supporting MAS1 receptors coupling to Ang(1–7), attempts at pharmacological analysis of MAS1 receptors in tissue membrane preparations have shown that [<sup>125</sup>I]-Ang(1–7) binding is not specific. Furthermore, in competitive displacement experiments, Ang(1–7) is less potent than other angiotensin metabolites, leading to the suggestion that either the radioligand binding sites in tissue membrane preparations are non-specific or the isotope modifies Ang(1–7) in such a way as to affect the high-affinity interaction (Conti, 2016). Additional radioligand development could help resolve this problem.

Concentration-response studies in MAS1 as well as MAS1-GFP transfected cells have been used to estimate an IC<sub>50</sub> for the Ang(1–7) antagonist, D-Ala7-Ang(1–7) and K<sub>d</sub> values of [<sup>125</sup>I]-Ang(1–7) (Santos *et al.*, 2003; Gironacci *et al.*, 2011). The binding specificity of fluorescently labelled Ang(1–7) has been studied in CHO cells (Pinheiro *et al.*, 2004a,b; Savergnini *et al.*, 2010; Jankowski *et al.*, 2011), platelets (Fraga-Silva *et al.*, 2008) and Leydig cells (Leal *et al.*, 2009). Also the MAS1 receptor mediated signalling has been found to be stimulated by several other peptides such as NPFF, alamandine, Ang III, Ang IV, angiotensin, CGEN-857 and P61/P33 and CGEN-856. Some of the peptides have also been found to elicit a response through the MRGPRD receptor, but additional systematic studies are warranted (Dong *et al.*, 2001; Bikkavilli *et al.*, 2006; Canals *et al.*, 2006; Gembardt *et al.*, 2008; Shemesh *et al.*, 2008; Savergnini *et al.*, 2010; Jankowski *et al.*, 2011; Zhang *et al.*, 2012; Savergnini *et al.*, 2013; Tirupula *et al.*, 2014a; Lee *et al.*, 2015). In transfected HEK293 cells, the MAS1 receptor was found to activate G<sub>q</sub>/11-PLC signalling without ligand stimulation (Canals *et al.*, 2006). Several ligands that modulate MAS1 receptor

signalling by other G-proteins and receptor desensitization have been described. These include peptide ligands such as MBP7 (Bikkavilli *et al.*, 2006), NPFF, AngIII, AngIV and the small nonpeptide agonist AR234960 and inverse agonist AR244555 (Zhang *et al.*, 2011; Tirupula *et al.*, 2014a). G protein mediated cAMP signalling by MAS1 and MRGPRD receptors in response to stimulation by peptides and non-peptide ligands have been recently reported (Tirupula *et al.*, 2014a; Klein *et al.*, 2015; Tetzner *et al.*, 2016). Ang(1–7) has been shown to increase arachidonic acid levels but, it does not modulate G<sub>q</sub>-PLC signalling in MAS1-expressing cells (Bikkavilli *et al.*, 2006; Shemesh *et al.*, 2008; Zhang *et al.*, 2011; Tirupula *et al.*, 2014a) leading to the concept of functional selectivity of ligands interacting with MAS1 receptors (Tirupula *et al.*, 2014a).

## Signalling

Ang(1–7)- or AVE0991-activated MAS1 receptors have been demonstrated to couple to many downstream signalling pathways (see Figure 2) including activation of phospholipase A<sub>2</sub>, release of arachidonic acid, calcium-independent activation of NOS, activation of phosphatidylinositol 3-kinase/Akt, MAP kinases, RhoA, and cAMP/PKA in human mesangial cells and MAS1 transfected CHO and COS cells (Santos *et al.*, 2003; Sampaio *et al.*, 2007a,b; Gembardt *et al.*, 2008; Zimpelmann and Burns, 2009; Liu *et al.*, 2011; Lopez Verrilli *et al.*, 2011; Verano-Braga *et al.*, 2012; Savergnini *et al.*, 2013; Than *et al.*, 2013). In early studies in MAS1 transfected cells, treatment with Ang(1–7) or AVE099 did not produce a G protein coupled calcium, IP<sub>3</sub> and cAMP signalling response (Bikkavilli *et al.*, 2006; Shemesh *et al.*, 2008). However, this observation was disputed by findings in the kidney, where Ang(1–7) treatment increased cAMP levels and activated PKA through G<sub>αs</sub> activation by MAS1 receptors. These signals could be inhibited using A-779 (Magaldi *et al.*, 2003; Liu *et al.*, 2011). The MAS1 receptor was also shown to constitutively couple to G<sub>αi</sub>, G<sub>αq</sub> and G<sub>α12/13</sub> proteins (Zohn *et al.*, 1998; Whitehead *et al.*, 2001; Booden *et al.*, 2002; Chen and Ikeda, 2004; Dias-Peixoto *et al.*, 2008; Shemesh *et al.*, 2008; Singh *et al.*, 2010; Zhang *et al.*, 2011; Gomes *et al.*, 2012; Tirupula *et al.*, 2014a). Conventional G protein signalling by MAS1 receptors was reported upon stimulation with neuropeptide FF (Dong *et al.*, 2001; Tirupula *et al.*, 2014a), MBP7 (Bikkavilli *et al.*, 2006), the angiotensin metabolites, AngIII and AngIV (Gembardt *et al.*, 2008; Tirupula *et al.*, 2014a), novel non-peptide ligands, AR234960, AR244555 and AR305352 (Zhang *et al.*, 2011; Tirupula *et al.*, 2014a) and CGEN-856/P61 (Shemesh *et al.*, 2008; Savergnini *et al.*, 2010). Thus, this atypical G protein response may be due to the functional selectivity of the MAS1 receptor, which allows different ligands to activate different signalling pathways (Tirupula *et al.*, 2014a). Calcium-independent activation of NOS, and Gi/o-mediated cAMP signalling is reported for Ang(1–7) engaged with AT<sub>2</sub> and MRGPRD receptors. However, additional validation studies are required to elucidate the ligand selectivity of different modes of signalling in these receptors.

Heterodimeric interactions of AT<sub>1</sub> and MAS1 receptors was reported in transfected cells. MAS1-AT<sub>1</sub> receptor hetero oligomerization resulted in the altered trafficking of AT<sub>1</sub>



receptors in transfected cells (Kostenis *et al.*, 2005; Canals *et al.*, 2006; Santos *et al.*, 2007). The physiological effects of Ang(1–7) treatment in mice may involve MAS1 receptors interacting with AT<sub>1</sub> and AT<sub>2</sub> receptors (Castro *et al.*, 2005). The MAS1 receptor was shown to function as a physiological antagonist of the AT<sub>1</sub> receptor by altering the response to AngII in the mice (Von Bohlen und Halbach *et al.*, 2000; Kostenis *et al.*, 2005; Rakusan *et al.*, 2010). Thus, the interaction of MAS1 receptors with AT<sub>1</sub> and AT<sub>2</sub> receptors may be a mechanism for the MAS1 receptor in RAS pathophysiology (Vilella *et al.*, 2014a). In experimental disease models Ang(1–7)/MAS1 signalling suppress AngII-induced pathogenesis. For example, AngII-induced ROS overproduction and apoptosis in cerebral endothelial cells (Xiao *et al.*, 2015), phosphorylation of c-Src and MAPKs, leading to production of TGF- $\beta$ 1 and collagen in cardiac fibroblasts (Meng *et al.*, 2014; Tao *et al.*, 2014; Zheng *et al.*, 2015a), muscle atrophy of gastrocnemius (Cisternas *et al.*, 2014), pancreatic cell damage (Wang *et al.*, 2014), ICAM-1, VCAM-1, and MCP-1 expression in HUVECs (Liang *et al.*, 2015a,b) is suppressed by Ang(1–7) activated MAS1 receptors.

Similarly, receptor heterodimer dependent crosstalk between the bradykinin B<sub>2</sub> receptor and MAS1 receptor was implicated in vasorelaxation responses. Microvessels in MAS1-deficient mice lacked the capacity to relax in response to both Ang(1–7) and bradykinin (Peiro *et al.*, 2013). Fluorescence resonance energy transfer analysis showed that the B<sub>2</sub> receptor and MAS receptor formed a constitutive heteromer, leading to potentiation of agonist binding to both receptors. B<sub>2</sub> receptor or MAS1 receptor antagonists promoted heteromer dissociation. Agonist stimulation promoted internalization of the heteromer into early endosomes. B<sub>2</sub> receptor-MAS1 receptor heteromers have been shown in human glomerular endothelial cells by proximity ligation assay (Cerrato *et al.*, 2016).

G protein independent signalling scaffold proteins have been shown to interact with the MAS receptor C-terminal tail in a tissue specific manner (Tirupula *et al.*, 2015). This mechanism may explain the signalling events that were demonstrated to be not associated with the generation of G protein mediated second messengers. The MAS receptor has been shown to interact with postsynaptic density 95 (PSD95), a novel binding protein. MAS interacts with the PDZ1–2 domain of PSD95 through engaging the last four amino acids [ETVV (Glu-Thr-Val-Val)] of MAS-CT. This interaction with PSD95 enhanced the expression and proteolysis stability of MAS receptors in cells (Bian *et al.*, 2013). An agonist driven 'MAS-signalosome' model has also been proposed as molecular mechanism of MAS receptor function. Identifying the hierarchy of interactions of 'signalosome' components with MAS receptors will be a necessary step in the future to fully understand the physiological and pathological functions of this enigmatic receptor (Tirupula *et al.*, 2015).

## Conclusion

Initially, the pairing of Ang(1–7) with the MAS1 receptor generated huge excitement, in part, due to the lack of a reasonable alternative target for Ang(1–7) at the time. However, now three Ang(1–7) receptor candidates have been

proposed by independent groups, all of which meet some of the criteria used by IUPHAR in their de-orphanization efforts. Firstly, the findings that Ang(1–7) stimulates the MAS1, MRGPRD and AT<sub>2</sub> receptors with potencies consistent with physiological function have been independently established. Secondly, the distribution of ACE2 – the enzyme producing Ang(1–7) at physiologically significant concentrations – is anatomically co-localized with endogenous MAS1, MRGPRD and AT<sub>2</sub> receptors, which supports the local production and action of Ang(1–7). Thirdly, genetic modification affecting each of these three receptors alters the physiological response to Ang(1–7) in experimental models. The Ang(1–7)/MAS1 receptor axis currently seems to be most studied beneficial counter-regulator of the effects of activation of the renin-angiotensin system in several neurological and cardiovascular diseases. In comparison, the Ang(1–7)/MRGPRD and Ang(1–7)/AT<sub>2</sub> receptor pairing require extensive further evaluation. A recent paper has proposed an Ang(1–7)/AT<sub>1</sub> receptor biased signalling mechanism (Galandrin *et al.*, 2016), but this needs independent confirmation. Improving the validity of the pharmacological tests, including saturation, competitive radioligand binding and signalling assays, which may further support and strengthen the Ang(1–7) pairing with each of these GPCRs would be most helpful. The criteria that selective agonists should mimic and antagonists should selectively block the signals of the endogenous ligand through each of these GPCRs are not uniformly established for all putative ligands. The recent discovery of an AT<sub>2</sub> receptor-selective antagonist PD123319, which inhibits the action of Ang(1–7) mediated through MAS1 and MRGPRD receptors, is a case in point for the confusing state of molecular pharmacology of Ang(1–7) receptors. However, the existence of multiple receptors responding to Ang(1–7) may have evolved for tissue-specific sensitive effects. A specific Ang(1–7) receptor axis may mediate a particular effect, which depends on the concentration of one or other of the receptors in a tissue. Until the unique distinctions of each receptor pairing with Ang(1–7) emerges, the adoption of these receptors strictly as Ang(1–7) receptors by the nomenclature committee may well be provisional.

## Acknowledgements

This work was supported in part by National Institutes of Health Grants, HL115964 and HL132351 to S. K. and National Research Service Award, HL007914 to H. U.

## Conflict of interest

The authors declare no conflicts of interest.

## Methodology

### Compiler note

We performed a systematic computerized literature search of the PubMed database, Scopus and Web of Science (last search: 16 September 2016) to identify all published articles on MAS1 from 1 January 2013 to 16 September 2016. The syntax/key

words used in the literature search are MAS1 or MAS1 receptor or Ang(1–7) receptor. The reference lists all articles we recovered and those of relevant review articles were also cross-referenced.

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