AP&T Alimentary Pharmacology and Therapeutics

Review article: the pathophysiological roles of the renin–angiotensin system in the gastrointestinal tract

M. Garg[*,§], P. W. Angus[†,‡], L. M. Burrell[†], C. Herath[†], P. R. Gibson[*,§] & J. S. Lubel[*,‡,§]

[*]Department of Gastroenterology & Hepatology, Eastern Health, Vic., Australia.
[†]Department of Medicine, Melbourne University, Vic., Australia.
[‡]Gastroenterology and Liver Transplant Unit, Austin Hospital, Vic., Australia.
[§]Eastern Health Clinical School, Monash University, Vic., Australia.

Correspondence to:
Dr M. Garg, Department of Gastroenterology & Hepatology, Eastern Health, Level 2, 5 Arnold St, Box Hill 3128, Vic., Australia.
E-mail: mayur.garg@monash.edu

Publication data
Submitted 24 October 2011
First decision 29 November 2011
Resubmitted 12 December 2011
Accepted 13 December 2011
EV Pub Online 5 January 2012

This uncommissioned review article was subject to full peer-review.

SUMMARY

Background
The renin–angiotensin system (RAS) is a homeostatic pathway widely known to regulate cardiovascular and renal physiology; however, little is known about its influence in gastrointestinal tissues.

Aim
To elicit the anatomical distribution and physiological significance of the components of the RAS in the gastrointestinal tract.

Methods
An extensive online literature review including Pubmed and Medline.

Results
There is evidence for RAS involvement in gastrointestinal physiology and pathophysiology, with all the components required for autonomous regulation identified throughout the gastrointestinal tract. The RAS is implicated in the regulation of glucose, amino acid, fluid and electrolyte absorption and secretion, motility, inflammation, blood flow and possibly malignant disease within the gastrointestinal tract. Animal studies investigating the effects of RAS blockade in a range of conditions including inflammatory bowel disease, functional gut disorders, gastrointestinal malignancy and even intestinal ischaemia have been encouraging to date. Given the ready availability of drugs that modify the RAS and their excellent safety profile, an opportunity exists for investigation of their possible therapeutic role in a variety of human gastrointestinal diseases.

Conclusions
The gastrointestinal renin–angiotensin system appears to be intricately involved in a number of physiological processes, and provides a possible target for novel investigative and therapeutic approaches.

Aliment Pharmacol Ther 2012; **35:** 414–428

© 2012 Blackwell Publishing Ltd
doi:10.1111/j.1365-2036.2011.04971.x

INTRODUCTION

The renin–angiotensin system (RAS) plays a central role in regulating cardiovascular and renal physiology. The contemporary view of the RAS has evolved from that of a simple linear pathway involving the conversion of angiotensinogen to angiotensin II (Ang II) via a two-step process facilitated by renin and angiotensin converting enzyme (ACE), to a much more complex system involving homologues of ACE and multiple angiotensin peptides which play supplementary and counter-regulatory roles (Figure 1). The RAS was, for many years, thought of as an endocrine system with enzymes and peptides released into the systemic circulation to act on target organs. More recently, it has been recognised that most organs including the brain, kidney, heart, liver, pancreas, reproductive organs, skin and the gastrointestinal tract constitutively express all the components required to allow autonomous function of a local intra-organ RAS, where it performs both paracrine and autocrine functions.

UNDERSTANDING THE COMPONENTS OF THE RAS – OLD AND NEW

Table 1 summarises the current view of the RAS, the key components and their physiological and clinical effects. Essentially, the relative activity of two counterbalancing pathways determines the predominant tissue effect.

The proinflammatory, profibrotic pathway includes the classical RAS components ACE and Ang II, and renin, prorenin, chymase and neutral endopeptidase (NEP, also known as neprilysin). Renin, a glycoprotein derived predominantly from the juxtaglomerular apparatus in the kidney, is an aspartyl protease that cleaves the liver-derived angiotensinogen to angiotensin I. Both renin and its proenzyme prorenin, which was previously considered physiologically inactive,[1] have now been demonstrated to have independent pro-inflammatory and pro-fibrotic effects via signalling through the pro(renin) receptor (PRR).[2]

The classical RAS comprising the zinc metalloproteinase ACE and Ang II induces vasoconstriction, salt and

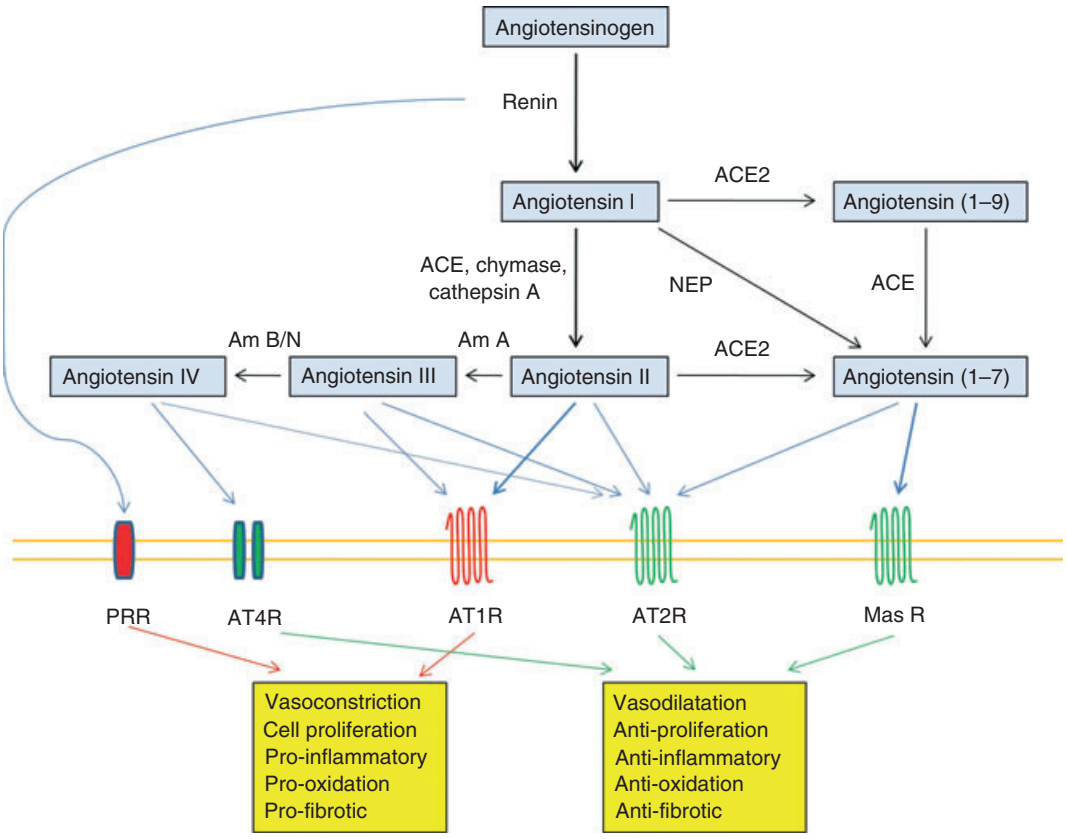

Figure 1 | The contemporary renin–angiotensin system (RAS). ACE, angiotensin converting enzyme; NEP, neutral endopeptidase; Am, aminopeptidase; AT1R, angiotensin type 1 receptor; AT2R, angiotensin type 2 receptor; AT4R, angiotensin type 4 receptor; PRR, (pro)renin receptor.

© 2012 Blackwell Publishing Ltd

Table 1 | RAS components and their molecular and physiological effects

RAS components	Mediating receptor	Molecular signals identified	Predominant physiological and clinical effects
Renin and prorenin	(Pro)renin receptor (PRR)	Activates extracellular signal-regulated kinases (ERK) 1/2, TGFβ, collagens, fibronectin, COX-2[2]	Diabetic microvascular complications, possibly cardiac and renal fibrosis[143]
Classical RAS components: ACE, Ang II	Angiotensin type 1 receptor (AT1R)	Activation of phospholipase C, mitogen-activated protein (MAP) kinase, initiation of NADPH oxidase, signal transducer and inhibitor of transcription (STAT) 1 activation, ubiquitination of IkB; leading to increased IL-6, TNFα, TGFβ, fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), insulin like growth factor (IGF)-1[144–148]	Vasoconstriction, salt and water retention, thirst response, cardiac hypertrophy, tissue inflammation and fibrosis
Alternative RAS components: ACE2, Ang (1–7)	*Mas* receptor	Inhibition of ERK1/2, MAP kinase, stimulation of nitric oxide (NO) release through endothelial nitric oxide syntetase, may directly antagonise the AT1R through heterodimerisation[149]	Vasodilatation, antihypertensive, anti-thrombotic, cardioprotective, anti-inflammatory and anti-fibrotic
Angiotensin III	Angiotensin type 1 receptor (AT1R)	Increases monocyte chemoattractant protein (MCP-1), NFκB and activating protein-1 (AP-1) activity in renalmesangialcells[150]; aldosterone secretion from adrenalglands[151]	Proinflammatory, possibly renal fibrosis
Angiotensin IV	Angiotensin type II receptor (AT2R) Angiotensin type 4 receptor (AT4R)	Inhibition of tyrosine kinase/STAT signalling pathway and NFκB stimulates nitric oxide production, may directly antagonise the AT1R through heterodimerisation[152–155]	Anti-inflammatory, central nervous system effects (neuronal development, learning and memory)
Chymase		May convert Ang I to Ang II, activates TGFβ and MMP-9[156]	Cardiac and vascular fibrosis
Neural endopeptidase (NEP, neprilysin)		Converts Ang I to Ang (1–7), inactivates atrial natriuretic peptideand kinins,[9] may degrade amyloid β peptide[10]	Vasoconstriction, antidiuresis, hypertension[11]

ACE, angiotensin converting enzyme; Ang II, angiotensin II; RAS, renin–angiotensin system.

© 2012 Blackwell Publishing Ltd

water retention, thirst response, cardiac hypertrophy, tissue inflammation and fibrosis through the G-protein coupled seven-transmembrane domain receptor angiotensin type I receptor (AT1R). Ang II also stimulates adrenal gland secretion of aldosterone resulting in renal sodium and water retention. Inhibition of this pathway with either ACE inhibitors or AT1R antagonists has beneficial effects in hypertension, cardiac failure, ischaemic heart disease, diabetic nephropathy and renal fibrosis.

Chymase expressed in the heart and vascular wall and secreted by activated mast cells, acts as an alternative enzyme to ACE to generate Ang II from Ang I.[3–6] NEP, a membrane bound zinc metalloproteinase with a structure distinct from ACE, was discovered in the 1970s as a key enzyme involved in the cleavage of bradykinin.[7, 8] In recent years, it has been shown to also have a role in the formation of Ang (1–7) from Ang I, as an inactivator of atrial natriuretic peptide[9] and in the degradation of amyloid β peptide,[10] a protein involved in the pathogenesis of Alzheimer's disease. The net effect of NEP inhibition is vasodilatation and natriuresis, a property encompassed by vasopeptidase inhibitors that target both ACE and NEP and may have additional anti-hypertensive effects to ACE inhibitors.[11]

In contrast, the alternative RAS, comprising ACE2 and Ang (1–7), acting via the G-protein coupled seven-transmembrane receptor *mas*,[12, 13] has vasodilatory, antihypertensive, anti-thrombotic, cardioprotective, anti-inflammatory and anti-fibrotic effects in a variety of tissues.[14–18] ACE2 is a zinc metalloproteinase and homologue of ACE, which cleaves a single amino acid from Ang II to form the heptapeptide Ang (1–7). Indeed, part of the clinical benefit attributed to ACE inhibitors and AT1R blockers (ARBs) may be through the diversion of the classical RAS components towards Ang (1–7) with subsequent *mas* receptor activation.[18–20]

The complexity of the RAS is further highlighted by recent findings regarding the actions of other angiotensin peptides including angiotensin III [Ang III, also denoted as Ang (2–8)], angiotensin IV [Ang IV, also known as Ang (3–8)] and the AT2 and AT4 receptors. Ang III is formed by cleavage of Ang II by aminopeptidase A, and Ang IV results from further conversion by aminopeptidase B or N (Figure 1).

The AT2 receptor (AT2R) has affinity for Ang II, Ang III, Ang IV and Ang (1–7), and is also thought to have effects counteracting the AT1R and analogous to those of the *mas* receptor, with vasodilatory, anti-inflammatory and anti-proliferative downstream actions. Previously recognised largely for an important role in foetal development, more recently, the AT2R has been shown to be upregulated in atherosclerotic disease,[21] cutaneous wounds[22] and pancreatic fibrosis,[23] and to stimulate neurite outgrowth, a marker of neuronal regeneration.[24, 25]

Ang III is believed to have actions analogous to Ang II. Ang IV appears to have opposing effects to Ang II, and acts predominantly via the AT2R and the AT4R, formerly known as insulin regulated aminopeptidase (IRAP).[26] The greatest role of Ang IV is in the central nervous system (CNS), where it has a positive effect on neuronal development, learning and memory.[27, 28]

CONCEPT OF LOCAL RAS

There is considerable evidence that most or all of the components of the RAS are present in a variety of organs, supporting the theory that local expression and modulation of the RAS play important roles in tissue homeostasis. These roles may be summarised as involving (1) fluid and electrolyte transport, (2) regional blood flow regulation and (3) promoting the wound healing response, including cell proliferation, inflammation and fibrosis. Some of the regional effects of the RAS are listed below:

(i) In the kidney, local angiotensinogen is converted by renin to Ang I, which in turn is cleaved by tubular brush border ACE to Ang II to facilitate sodium and fluid absorption via luminal AT1R. This may influence blood pressure independent of systemic Ang II levels and vascular tone.[29, 30]

(ii) The heart expresses renin, PRR, ACE, chymase, angiotensinogen, AT1R and AT2R, and these components modulate myocyte proliferation and cardiac remodelling.[6, 31–37]

(iii) The brain has been shown to express renin, angiotensinogen, Ang II, Ang III, Ang IV, Ang (1–7), AT1R, AT2R and AT4R, with these components regulating blood pressure, fluid and electrolyte balance, thirst, maintenance of the blood-brain barrier and neuronal development including learning and memory processes.[38–44]

(iv) The liver expresses renin, angiotensinogen, Ang II, ACE, AT1R, Ang (1–7), ACE2 and *mas* receptors, all of which are upregulated in the diseased liver.[45, 46] Furthermore, ARBs and Ang (1–7) have been demonstrated to reduce liver fibrosis in animal models.[47–49]

(v) In the pancreas, Ang II has been shown to inhibit glucose stimulated insulin secretion, and via AT1R and AT2R, regulates exocrine enzyme secretion and the microcirculation.[50–52]

© 2012 Blackwell Publishing Ltd

(vi) A local RAS has been identified and shown to be involved in tissue homeostasis in the reproductive organs, skin and even adipose tissue.[53–58] Detailed reviews of these and other local RAS effects are published elsewhere.[12, 30, 50, 54, 59–61]

LOCALISATION AND FUNCTIONALITY OF THE RAS IN THE GASTROINTESTINAL TRACT

Our understanding of the involvement of the RAS in the gastrointestinal tract has gradually evolved over the past five decades since the formulation of the hypothesis that Ang II had a direct effect on intestinal smooth muscle in addition to an indirect effect via myenteric plexus cholinergic neurons.[62–64] Since then, many of the components of the RAS have been identified throughout the gastrointestinal tract. An overview of the current state of knowledge is illustrated in Figure 2. The regions of the gut are addressed separately here.

Small intestine

Most attention has been paid to the small intestine (Figure 2a), as outlined below:

(i) *ACE, ACE2 and neutral endopeptidase:* ACE has been shown in humans to be located in abundance on the brush border of epithelial cells and in the mesenteric microvascular endothelium.[65] ACE2 mRNA and protein is present in large amounts in small intestinal epithelial brush border, muscularis mucosa and muscularis propria, as well as microvascular endothelium and vascular smooth muscle cells.[66] Remarkably, the highest tissue concentrations in the human body of ACE and ACE2 mRNA are found in the terminal ileum, duodenum and colon.[67, 68] Expression of NEP has been demonstrated in the rat intestinal wall, and is suppressible by administration of the combined ACE/NEP inhibitor omapatrilat.[69]

(ii) *Angiotensin receptors:* AT1R has been localised to the epithelial brush border.[70] The circular and longitudinal muscle layers and the myenteric plexus also strongly express AT1R, but the AT2R appears to be largely restricted to the myenteric plexus.[71, 72] Small vessels in the muscularis propria also express AT1R.[71, 72] In early studies in the rat intestine, Ang II binding sites were reported to be confined to the muscularis,[73] but subsequent reports have identified expression of AT1R and a lesser amount of AT2R in the muscularis mucosa and mucosa, including in epithelial cells.[74, 75]

(iii) *Renin:* mRNA for renin has been detected in the human small intestine.[76]

(iv) *Angiotensin peptides:* Ang II has been detected in the crypt and crypt-villus junction epithelial cells.[70] To date, the expression of angiotensinogen, Ang I or Ang (1–7) in the human small intestine has not been reported. However, angiotensinogen has been widely localised in the rat brush border, epithelial cells, lamina propria, muscularis mucosa, submucosal blood vessels and muscularis propria.[77] Angiotensinogen mRNA has been isolated in concentrations of over one-third that of the liver in the rat mesentery,[78] and a high level of pro-angiotensin-12, a precursor of Ang I, has been located in the rat intestine.[79]

Thus, all of the required components for local production and action of Ang II appear to be present in small intestine.

There is now evidence of important roles for the RAS in a variety of intestinal processes:

(i) *Bicarbonate secretion:* This is stimulated by Ang II via AT1R and AT2R in the duodenum.[80]

(ii) *Sodium and water absorption:* In the jejunum and ileum, this process appears to be modulated by Ang II in conjunction with the enteric sympathetic nervous system.[81–83] When applied in low dose to rat jejunum, Ang II stimulates sodium and water absorption through AT2R, but in high dose, it unexpectedly inhibits absorption through AT1R.[84] Both Ang II and Ang III may also increase sodium and water absorption via stimulation of release of noradrenaline from sympathetic neurons, which in turn may act through adrenergic receptors on the basal surface of epithelial cells.[83, 85, 86]

(iii) *Glucose absorption:* Ang II has also been shown to inhibit rat jejunal sodium-dependent glucose transporter (SGLT1)-mediated glucose uptake *in vitro*.[77]

(iv) *Digestion and absorption of peptides:* Both brush border ACE and ACE2 are thought to function as peptidases, allowing for mucosal digestion and absorption of peptides.[87, 88] ACE2 increases the activity of the neutral amino acid transporter B^0AT1, which is mutated in a rare amino acid deficiency disorder, Hartnup disorder, clinically manifested by cerebellar ataxia and pellagra-like skin rash.[89]

(v) *Secretion:* A role for ACE2 in active secretion has been suggested by the observation that ACE2 is the target for the coronavirus mediating severe acute respiratory syndrome, SARS-CoV. Some patients with this infection suffer from watery diarrhoea, but the exact mechanism remains to be determined.[66]

© 2012 Blackwell Publishing Ltd

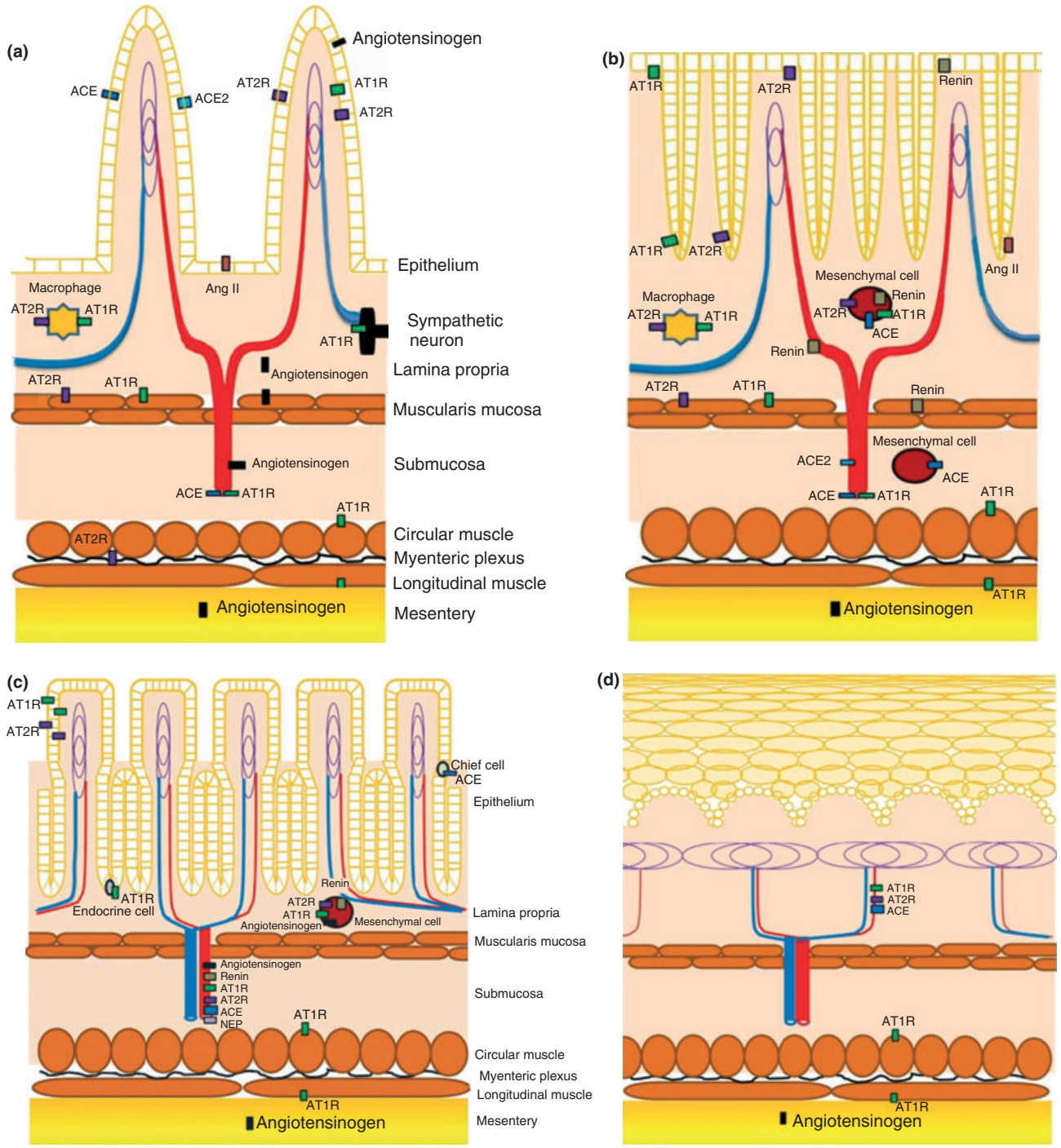

Figure 2 | Distribution of components of the RAS in the gastrointestinal tract. Diagrammatic representation of RAS components in (a) small intestine, (b) colon, (c) stomach and (d) oesophagus.

Colon

There have been limited studies of RAS components in the colonic wall (Figure 2b). By a combination of RT-PCR and immunohistochemistry, renin was found in the surface epithelium, lamina propria mesenchymal cells, microvascular walls and muscularis mucosa. AT1R was detected on surface epithelial cells and in crypt bases, lamina propria macrophages, myofibroblasts and mucosal vessel walls, and weak expression of AT2R has been found on surface epithelium, in crypts and in some mesenchymal cells.[90] ACE was also weakly expressed in parts of the surface epithelium, and more prominently in

© 2012 Blackwell Publishing Ltd

mesenteric microvascular walls, lamina propria and submucosal mesenchymal cells.[90] ACE2 appears to be localised to the mesenteric microvascular endothelium in the colon and is not present in the epithelium.[66] Angiotensinogen mRNA has also been isolated in homogenised rat colon,[78] but its expression has not been examined in the human colon.

A more limited range of functional roles has been attributed to components of the RAS in the colon than in small intestine. Ang II has been shown to increase sodium and water reabsorption in rats through NaCl coupled transport.[91] The response of circular and longitudinal muscle contraction to Ang II also suggests a role in normal colonic motility.[92] As detailed below, the RAS may also be involved in the inflammation associated with IBD, as mucosal levels of Ang I and Ang II are higher in patients with active Crohn's colitis compared with normal controls and patients with ulcerative colitis.[93]

Stomach

Components of the RAS are present in the mucosal biopsy specimens of gastric antrum and body from healthy adults (Figure 2c).[94] Renin and angiotensinogen were both seen in lamina propria mesenchymal cells and vascular endothelial cells. AT1R and AT2R were both observed in gastric epithelium (mainly in the basal surface), lamina propria mesenchymal cells and vascular endothelium. AT1R were noted in a subgroup of endocrine cells in the base of antral mucosal glands, and ACE and NEP in vascular endothelial cells, but not in other parts of the mucosa. Other investigators, however, have noted ACE in fundic chief cells and mucin secreting cells of the antrum.[95] Longitudinal and circular muscle of the stomach has been demonstrated to respond *in vitro* to Ang II, suggesting the presence of appropriate receptors on gastric myocytes.[96]

To date, few functional or pathogenic roles have been attributed to the RAS in the stomach. A role of local RAS in gastric inflammation has been suggested by higher expression of AT1R expression in *Helicobacter pylori* positive than *H. pylori* negative patients[94] and the potentiation of ulceration in animal models by Ang II.[97, 98]

Oesophagus

Immunoreactive ACE, AT1R and AT2R have been found in the lamina propria microvascular walls, and AT1R and AT2R were identified in the superficial stratified epithelium[99] and circular and longitudinal muscle of the oesophagus[100] (Figure 2d). Ang II caused contraction of isolated oesophageal smooth muscle *in vitro*, and the AT1R antagonist candesartan inhibited swallow-induced peristaltic contractions in the distal oesophagus.[100] The expression of other RAS components has not been reported.

POTENTIAL CLINICAL IMPLICATIONS OF RAS IN GASTROINTESTINAL DISEASE

The presence of the various components of the RAS in the gastrointestinal tract raise the possibility that modification of this system locally may be a potential therapeutic target in a myriad of gastrointestinal diseases where current strategies are suboptimal. These include inflammatory bowel disease (IBD), gastrointestinal cancer, gut motility disorders and mesenteric ischaemia. Although clinical data are sparse, results from animal models and pre-clinical studies provide support for further investigation.

Inflammatory bowel disease

Information relevant to IBD has arisen from studies in Crohn's disease (CD) and ulcerative colitis (UC) and animal models of both IBD and other chronic inflammatory conditions.

Studies in patients with Crohn's disease and ulcerative colitis. Two components of the RAS have been studied. The first is ACE, which was subject to intense interest from the 1980's in its role in sarcoidosis and other granulomatous conditions. Studies of serum ACE concentrations yielded largely conflicting findings in IBD, with many studies showing reduced levels[101–104] and some finding no difference.[105–107] Many of these studies have been limited by relatively small numbers of patients.

Serum ACE levels are associated with ACE gene insertion/deletion (I/D) polymorphisms, with higher levels seen with the DD polymorphism than ID or II.[108] Matsuda *et al.* showed that the ACE gene polymorphism variation was similar in 39 patients with CD and 43 patients with UC to 341 controls, but that serum ACE levels were lower in patients with IBD after adjusting for polymorphisms.[101] Furthermore, they demonstrated that ACE levels significantly increased in all of nine patients with active CD when they achieved clinical remission. A larger study involving 124 UC patients and 108 CD patients also found no difference in ACE gene polymorphisms when compared with normal controls, but a subgroup analysis revealed a higher proportion of DD genotype in 25 UC patients with extra-intestinal manifestations, with

© 2012 Blackwell Publishing Ltd

an odds ratio (OR) of 4.08.[109] Indeed, it is difficult to reconcile these findings into a unifying hypothesis regarding the role of ACE in IBD pathogenesis. It is possible that inflammatory cytokines such as TNF-α and IL-1 downregulate systemic endothelial ACE production,[110] whereas Ang II produced via local intestinal ACE contributes to tissue inflammation. Tissue ACE levels have not been measured in active IBD.

The other major area of investigation has focussed on angiotensin peptide levels in CD. Genotype analyses have revealed a significant association of angiotensinogen-6 AA genotype (OR 2.38) in a cohort of 235 patients with IBD. This genotype results in increased production of angiotensinogen via a substitution in its gene promoter.[111] Indeed, mucosal levels of Ang I and Ang II are elevated in rectosigmoid biopsies in patients with Crohn's colitis compared with patients with UC or normal controls,[93] and a significant correlation was noted between these levels and endoscopic grade of colitis. Although these findings demonstrate that components of the local tissue RAS probably play a part in inflammation, they do not prove any causal link in the pathogenesis of IBD. Nonetheless, they suggest that inhibition of the local RAS provides a potential avenue for targeting inflammation and fibrosis.

Studies in animal models. Angiotensin converting enzyme inhibition or angiotensin receptor antagonism have been shown to produce a number of beneficial anti-inflammatory effects in rodent models of intestinal inflammation. Ang II and AT1a receptor expression are both upregulated in dextran sodium sulphate (DSS)-induced colitis, a widely studied mouse model of colitis, and inflammation was significantly ameliorated in AT1a receptor-deficient mice.[112] The ACE inhibitor enalaprilat, given parenterally, reduced inflammation and TNF-α,[113] and topical enalaprilat reduced TGF-β expression and fibrosis in mice with DSS-colitis.[114] Administration of the ARB, valsartan, significantly reduced macroscopic inflammation, TNF-α, TGF-β and IL-18 in mice with trinitrobenzene sulphonic acid (TNBS) induced colitis, reduced microscopic inflammation and raised IL-10 (an anti-inflammatory cytokine secreted by regulatory T cells) in DSS-colitis.[115, 116] The ACE inhibitor captopril reduced macroscopic and microscopic inflammation, fibrosis and TGFβ mRNA expression in mice with TNBS-induced colitis.[117] Homozygous deficiency of angiotensinogen protects against TNBS-induced colitis, with reduced IL1-β, IFN-γ and greater IL-4 and IL-10 production than wild-type mice.[116] Animal studies have not been restricted to the classical RAS pathway. NEP knockout mice have been shown to have more severe colitis in response to dinitrobenzene sulphonic acid than wild-type mice, which is prevented by the administration of recombinant NEP.[118]

Other evidence to suggest that RAS dysfunction may potentiate immune-based diseases such as IBD and provide a target for therapy comes from recent studies involving models for multiple sclerosis (MS). T helper type 1 (Th1) and type 17 (Th17) cells are strongly implicated in the pathogenesis of IBD, especially CD, and lisinopril and candesartan have been demonstrated to suppress Th1 and Th17 cytokine expression and induce Foxp3+ regulatory T cells in experimental autoimmune encephalitis (EAE), a mouse model of MS.[119] Other groups have shown a crucial role for Ang II and AT1R in EAE[59, 120] and murine autoimmune nephritis.[121]

Gastrointestinal cancers

The possible involvement of perturbed RAS components in solid organ malignancies represents a fascinating expansion of our insight into local tissue RAS. Early epidemiological studies demonstrated a reduced risk of incident and fatal cancer in patients on ACE inhibitors versus those on other anti-hypertensive medication.[122, 123] In contrast, a recent meta-analysis reported a higher risk of lung cancer in patients taking ARBs[124]; however, two large meta-analyses since then have reported no increased risk.[125, 126] All these studies have been limited by their retrospective design.

Increasing *in vitro* and pre-clinical data suggest a protective effect of AT1R inhibition against cancer cell proliferation, invasion and metastasis in a variety of solid organs.[127] In gastric cancer, AT1R and Ang II are expressed to a greater extent than in adjacent normal tissue.[128] The AT1R and ACE gene polymorphism D allele increase risk of nodal metastasis and tumour stage in patients with intestinal-type gastric cancer.[129] In gastric cancer cell cultures, Ang II stimulates MAP kinase, NFκB and survivin activation, increasing proliferation.[128] The most likely mechanism by which the RAS may influence cancer biology is through increasing angiogenesis,[130, 131] via increased expression of vascular endothelial growth factor (VEFG) signalling.[130–132]

In mouse models, ACE inhibition and ARBs reduced colorectal cancer liver metastases [133] and prolonged survival in peritoneal carcinomatosis.[128] Indeed, ACE inhibitor use independently protected against distal metastasis in a single centre retrospective review of patients with colorectal carcinoma.[134] Also, patients treated with ACE inhibitors had a non-significant trend towards reduction

© 2012 Blackwell Publishing Ltd

in risk of oesophageal adenocarcinoma in a retrospective study of the UK general practice research database.[135]

Recently, there have been intriguing insights into the involvement of the RAS in Peutz-Jeghers Syndrome (PJS). Shorning and colleagues have shown that in mice LKB1 gene deletion, which is associated with PJS, results in marked transcriptional upregulation of the renin gene *Ren1*, and also increased ACE expression and Ang II production.[70] In human PJS tumour tissue, AT1R was noted to be increased in stromal tissue, but reduced in the epithelium.[70]

Gut motility disorders

The functional role of the RAS in smooth muscle contraction suggests that it might be a target in motility dysfunction. For example, the ability of Ang II blockade to inhibit contraction of oesophageal body and lower oesophageal sphincter (LES) smooth muscle, together with the demonstrated reduction in the amplitude of contraction of primary peristaltic oesophageal waves and LES on manometry *in vivo*,[100] suggests a possible role in treatment of hypercontractile oesophageal disorders such as diffuse oesophageal spasm, nutcracker oesophagus and achalasia. Furthermore, selective AT1R-mediated contraction of the LES may be an option for treatment of gastro-oesophageal reflux disease, a condition that affects up to 15–20% of the population.[136]

The contribution of AT1R to small and large intestinal muscle contractility also provides an opportunity to intervene in functional intestinal and motility disorders through AT1R agonism or blockade. Furthermore, the role of ACE and ACE2 in intestinal fluid and electrolyte absorption suggests a potential mechanism for modulating fluid shifts across the brush border, with subsequent effects on stool consistency and frequency.

Mesenteric ischaemia

The RAS plays an important role in regulation of the smooth muscle tone of the mesenteric vasculature. In acute hypovolaemia and systemic sepsis, splanchnic vasoconstriction occurs as a homeostatic response to preserve cerebral and renal blood flow, predisposing the gut to ischaemia. This splanchnic response has been shown to correlate with a markedly increased expression of Ang II.[137] Furthermore, lower-body negative pressure induction in normal human volunteers has also been shown to raise serum Ang II and reduce intestinal mucosal nitric oxide production.[138]

The administration of candesartan maintained jejunal and mucosal perfusion during severe hypovolaemia in

Table 2 | Potential therapeutic targets of the RAS

Mechanism of action	Drug/compound	Stage of development
ACE Inhibitors	Captopril	Clinical use
	Enalapril	Clinical use
	Fosinipril	Clinical use
	Lisinopril	Clinical use
	Perindopril	Clinical use
	Quinapril	Clinical use
	Ramipril	Clinical use
	Trandalopril	Clinical use
AT1 receptor blockers (ARB)	Azilsartan	Clinical use
	Candesartan	Clinical use
	Eprosartan	Clinical use
	Irbesartan	Clinical use
	Losartan	Clinical use
	Olmesartan	Clinical use
	Telmisartan	Clinical use
	Valsartan	Clinical use
AT2 receptor agonists	CGP42112A	*In vitro* studies
	Compound 21	Animal studies[21, 157]
	Compound 22	Animal studies[158]
AT2 receptor antagonists	Saralasin	Animal studies[159]
	PD123319	*In vitro* studies[24, 25]
AT4 receptor antagonists	Divalinal	Animal studies[160]
Ang (1–7)/Mas receptor agonists	Ang (1–7)	Animal studies[47, 161, 162]
	AVE 0991	Animal studies[47, 163–166]
Mas receptor antagonists	A-779	Animal studies[161]
ACE2 analogues	Recombinant hACE2 APN01	Animal studies[167, 168] Phase I Clinical trial
ACE2 inhibitors	C16	Animal studies [169]
NEP inhibitors	Omapatrilat (dual NEP and ACE inhibitor)	Withdrawn after clinical trials[170–172]
	LCZ696 (dual NEP and AT1R antagonist)	Phase 3 clinical trial[173]
(Pro)renin receptor antagonists	Handle region peptide (HRP)	Animal studies[174–180]
Direct renin inhibitors	Aliskiren	Clinical use

ACE, angiotensin converting enzyme; Ang II, angiotensin II; NEP, neutral endopeptidase; RAS, renin–angiotensin system.

© 2012 Blackwell Publishing Ltd

pigs and reduced mortality.[139, 140] A further porcine study reported improved mucosal oxygen delivery, but not an improvement in intestinal mucosal acidosis in pigs administered candesartan during endotoxic shock.[141] These results were replicated by Tardos *et al.* in pigs with burn and endotoxin induced gut ischaemia. In this model, intestinal permeability and bacterial translocation were reduced with the administration of the Ang II inhibitor DuP753.[142]

The obvious limiting factor in applying Ang II blockade in humans at risk of mesenteric ischaemia is the potential for current ARBs and ACE inhibitors to cause further hypotension and kidney injury, although no adverse renal consequences were noted in one porcine study.[139]

MANIPULATION OF LOCAL GASTROINTESTINAL TRACT RAS – POTENTIAL TARGETS AND LIMITATIONS

As a ubiquitous system with a wide array of homeostatic roles, investigation into therapies that manipulate the RAS has been extensive. Apart from the well-established roles of AT1 receptor blockade and ACE inhibition in the treatment of hypertension, cardiovascular and kidney disease, new drugs targeting the *mas* receptor, AT2 receptor, AT4R, renin and NEP, as well as a recombinant Ang (1–7), are under trial for various applications (Table 2).

There is limited knowledge of the effect of currently available ACE inhibitors and ARBs on gastrointestinal function at doses employed for cardiovascular and renal effect at a molecular level. Most of these drugs may result in adverse effects including nausea, diarrhoea or constipation in an idiosyncratic manner in between 1 and 3 per cent of patients, often noted in similar numbers of patients in the placebo arm in randomised controlled trials. It is likely that gastrointestinal tissue concentrations achieved by these medications at current doses are insufficient to note clinical effect. Furthermore, the effect of these drugs in pathological states, like functional gut disorders or IBD, has not been described.

For applicability to human gastrointestinal disease, drugs manipulating the RAS will need to target the relevant areas of the gut to obtain satisfactory tissue effect without systemic adverse reactions. Ideally, this will comprise delivery to the mucosa, absorption and binding of cellular receptors and sufficient first pass hepatic conversion to inactive metabolites to limit systemic effect.

CONCLUSIONS

There is now a significant body of literature demonstrating the existence and pathophysiological relevance of local tissue renin-angiotensin systems. Given the current evidence of involvement of the RAS in gastrointestinal fluid and electrolyte homeostasis, smooth muscle control, inflammation and malignancy, it follows that manipulation of this system could be of benefit in a range of GI pathologies. Therapies targeting the gastrointestinal RAS are attractive, given their excellent safety and tolerability profile and confirmed benefits in other organs and diseases. It is hoped that the recent emergence of further experimental evidence supporting a role for the local RAS in intestinal disorders will provide greater impetus for the initiation of well-conducted clinical trials in human disease.

ACKNOWLEDGEMENTS

Declaration of personal interests: None. *Declaration of funding interests*: Dr Mayur Garg is a recipient of the Gastroenterological Society of Australia Postgraduate Medical Research Scholarship.

REFERENCES

1. Nguyen G. Renin, (pro)renin and receptor: an update. *Clin Sci (Lond)* 2011; **120**: 169–78.
2. Nguyen G, Muller DN. The biology of the (pro)renin receptor. *J Am Soc Nephrol* 2010; **21**: 18–23.
3. Caughey GH, Raymond WW, Wolters PJ. Angiotensin II generation by mast cell alpha- and beta-chymases. *Biochim Biophys Acta* 2000; **1480**: 245–57.
4. Takai S, Shiota N, Sakaguchi M, Muraguchi H, Matsumura E, Miyazaki M. Characterization of chymase from human vascular tissues. *Clin Chim Acta* 1997; **265**: 13–20.
5. Takai S, Jin D, Sakaguchi M, Miyazaki M. Chymase-dependent angiotensin II formation in human vascular tissue. *Circulation* 1999; **100**: 654–8.
6. Urata H, Kinoshita A, Misono KS, Bumpus FM, Husain A. Identification of a highly specific chymase as the major angiotensin II-forming enzyme in the human heart. *J Biol Chem* 1990; **265**: 22348–57.
7. Kerr MA, Kenny AJ. The molecular weight and properties of a neutral metallo-endopeptidase from rabbit kidney brush border. *Biochem J* 1974; **137**: 489–95.

© 2012 Blackwell Publishing Ltd

8. Yang HY, Erdos EG, Levin Y. Characterization of a dipeptide hydrolase (kininase II: angiotensin I converting enzyme). *J Pharmacol Exp Ther* 1971; **177**: 291–300.
9. Troughton RW, Rademaker MT, Powell JD, *et al.* Beneficial renal and hemodynamic effects of omapatrilat in mild and severe heart failure. *Hypertension* 2000; **36**: 523–30.
10. Shirotani K, Tsubuki S, Iwata N, *et al.* Neprilysin degrades both amyloid beta peptides 1–40 and 1–42 most rapidly and efficiently among thiorphan- and phosphoramidon-sensitive endopeptidases. *J Biol Chem* 2001; **276**: 21895–901.
11. Skidgel RA, Erdos EG. Angiotensin converting enzyme (ACE) and neprilysin hydrolyze neuropeptides: a brief history, the beginning and follow-ups to early studies. *Peptides* 2004; **25**: 521–5.
12. Paul M, Poyan Mehr A, Kreutz R. Physiology of local renin-angiotensin systems. *Physiol Rev* 2006; **86**: 747–803.
13. Jackson TR, Blair LA, Marshall J, Goedert M, Hanley MR. The mas oncogene encodes an angiotensin receptor. *Nature* 1988; **335**: 437–40.
14. Freeman EJ, Chisolm GM, Ferrario CM, Tallant EA. Angiotensin-(1–7) inhibits vascular smooth muscle cell growth. *Hypertension* 1996; **28**: 104–8.
15. Ferreira AJ, Santos RA, Almeida AP. Angiotensin-(1–7) improves the post-ischemic function in isolated perfused rat hearts. *Braz J Med Biol Res* 2002; **35**: 1083–90.
16. Ferreira AJ, Santos RA, Almeida AP. Angiotensin-(1–7): cardioprotective effect in myocardial ischemia/reperfusion. *Hypertension*. 2001; **38**: 665–8.
17. Tallant EA, Ferrario CM, Gallagher PE. Angiotensin-(1–7) inhibits growth of cardiac myocytes through activation of the mas receptor. *Am J Physiol Heart Circ Physiol* 2005; **289**: H1560–6.
18. Chappell MC. Emerging evidence for a functional angiotensin-converting enzyme 2-angiotensin-(1–7)-MAS receptor axis: more than regulation of blood pressure? *Hypertension* 2007; **50**: 596–9.
19. Kucharewicz I, Chabielska E, Pawlak D, Matys T, Rolkowski R, Buczko W. The antithrombotic effect of angiotensin-(1–7) closely resembles that of losartan. *J Renin Angiotensin Aldosterone Syst* 2000; **1**: 268–72.
20. Kucharewicz I, Pawlak R, Matys T, Pawlak D, Buczko W. Antithrombotic effect of captopril and losartan is mediated by angiotensin-(1–7). *Hypertension* 2002; **40**: 774–9.
21. Kaschina E, Grzesiak A, Li J, *et al.* Angiotensin II type 2 receptor stimulation: a novel option of therapeutic interference with the renin-angiotensin system in myocardial infarction? *Circulation* 2008; **118**: 2523–32.
22. Steckelings UM, Henz BM, Wiehstutz S, Unger T, Artuc M. Differential expression of angiotensin receptors in human cutaneous wound healing. *Br J Dermatol* 2005; **153**: 887–93.
23. Ulmasov B, Xu Z, Tetri LH, Inagami T, Neuschwander-Tetri BA. Protective role of angiotensin II type 2 receptor signaling in a mouse model of pancreatic fibrosis. *Am J Physiol Gastrointest Liver Physiol* 2009; **296**: G284–94.
24. Meffert S, Stoll M, Steckelings UM, Bottari SP, Unger T. The angiotensin II AT2 receptor inhibits proliferation and promotes differentiation in PC12W cells. *Mol Cell Endocrinol* 1996; **122**: 59–67.
25. Laflamme L, Gasparo M, Gallo JM, Payet MD, Gallo-Payet N. Angiotensin II induction of neurite outgrowth by AT2 receptors in NG108-15 cells. Effect counteracted by the AT1 receptors. *J Biol Chem* 1996; **271**: 22729–35.
26. Numaguchi Y, Ishii M, Kubota R, *et al.* Ablation of angiotensin IV receptor attenuates hypofibrinolysis via PAI-1 downregulation and reduces occlusive arterial thrombosis. *Arterioscler Thromb Vasc Biol* 2009; **29**: 2102–8.
27. Karamyan VT, Speth RC. Enzymatic pathways of the brain renin-angiotensin system: unsolved problems and continuing challenges. *Regul Pept* 2007; **143**: 15–27.
28. von Bohlen, Halbach O. Angiotensin IV in the central nervous system. *Cell Tissue Res* 2003; **311**: 1–9.
29. Gurley SB, Riquier-Brison AD, Schnermann J, *et al.* AT(1A) angiotensin receptors in the renal proximal tubule regulate blood pressure. *Cell Metab* 2011; **13**: 469–75.
30. Lavoie JL, Sigmund CD. Minireview: overview of the renin-angiotensin system – an endocrine and paracrine system. *Endocrinology* 2003; **144**: 2179–83.
31. Hirsch AT, Talsness CE, Schunkert H, Paul M, Dzau VJ. Tissue-specific activation of cardiac angiotensin converting enzyme in experimental heart failure. *Circ Res* 1991; **69**: 475–82.
32. Nguyen G, Delarue F, Burckle C, Bouzhir L, Giller T, Sraer JD. Pivotal role of the renin/prorenin receptor in angiotensin II production and cellular responses to renin. *J Clin Invest* 2002; **109**: 1417–27.
33. Nguyen G, Burckle CA, Sraer JD. Renin/prorenin-receptor biochemistry and functional significance. *Curr Hypertens Rep* 2004; **6**: 129–32.
34. Paul M, Wagner J, Dzau VJ. Gene expression of the renin-angiotensin system in human tissues. Quantitative analysis by the polymerase chain reaction. *J Clin Invest* 1993; **91**: 2058–64.
35. Schelling P, Fischer H, Ganten D. Angiotensin and cell growth: a link to cardiovascular hypertrophy? *J Hypertens* 1991; **9**: 3–15.
36. Urata H, Healy B, Stewart RW, Bumpus FM, Husain A. Angiotensin II-forming pathways in normal and failing human hearts. *Circ Res* 1990; **66**: 883–90.
37. van Kesteren CA, van Heugten HA, Lamers JM, Saxena PR, Schalekamp MA, Danser AH. Angiotensin II-mediated growth and antigrowth effects in cultured neonatal rat cardiac myocytes and fibroblasts. *J Mol Cell Cardiol* 1997; **29**: 2147–57.
38. Ganten D, Minnich JL, Granger P, *et al.* Angiotensin-forming enzyme in brain tissue. *Science* 1971; **173**: 64–5.
39. Benarroch EE, Schmeichel AM. Immunohistochemical localization of the angiotensin II type 1 receptor in human hypothalamus and brainstem. *Brain Res* 1998; **812**: 292–6.
40. Rogerson FM, Schlawe I, Paxinos G, Chai SY, McKinley MJ, Mendelsohn FA. Localization of angiotensin converting enzyme by *in vitro* autoradiography in the rabbit brain. *J Chem Neuroanat* 1995; **8**: 227–43.
41. Campbell DJ, Bouhnik J, Menard J, Corvol P. Identity of angiotensinogen precursors of rat brain and liver. *Nature* 1984; **308**: 206–8.
42. Schiavone MT, Khosla MC, Ferrario CM. Angiotensin-[1–7]: evidence for novel actions in the brain. *J Cardiovasc Pharmacol* 1990; **16**(Suppl. 4): S19–24.
43. Wright JW, Harding JW. Important role for angiotensin III and IV in the brain renin-angiotensin system. *Brain Res Brain Res Rev* 1997; **25**: 96–124.
44. MacGregor DP, Murone C, Song K, Allen AM, Paxinos G, Mendelsohn FA. Angiotensin II receptor subtypes in the human central nervous system. *Brain Res* 1995; **675**: 231–40.
45. Herath CB, Warner FJ, Lubel JS, *et al.* Upregulation of hepatic angiotensin-converting enzyme 2

© 2012 Blackwell Publishing Ltd

(ACE2) and angiotensin-(1–7) levels in experimental biliary fibrosis. *J Hepatol* 2007; **47**: 387–95.
46. Paizis G, Cooper ME, Schembri JM, Tikellis C, Burrell LM, Angus PW. Up-regulation of components of the renin-angiotensin system in the bile duct-ligated rat liver. *Gastroenterology* 2002; **123**: 1667–76.
47. Lubel JS, Herath CB, Tchongue J, *et al.* Angiotensin-(1–7), an alternative metabolite of the renin-angiotensin system, is up-regulated in human liver disease and has antifibrotic activity in the bile-duct-ligated rat. *Clin Sci (Lond)* 2009; **117**: 375–86.
48. Paizis G, Gilbert RE, Cooper ME, *et al.* Effect of angiotensin II type 1 receptor blockade on experimental hepatic fibrogenesis. *J Hepatol* 2001; **35**: 376–85.
49. Jonsson JR, Clouston AD, Ando Y, *et al.* Angiotensin-converting enzyme inhibition attenuates the progression of rat hepatic fibrosis. *Gastroenterology* 2001; **121**: 148–55.
50. Leung PS, Chappell MC. A local pancreatic renin-angiotensin system: endocrine and exocrine roles. *Int J Biochem Cell Biol* 2003; **35**: 838–46.
51. Leung PS. Roles of the renin-angiotensin system and its blockade in pancreatic inflammation. *Int J Biochem Cell Biol* 2005; **37**: 237–8.
52. Skipworth JR, Szabadkai G, Olde Damink SW, Leung PS, Humphries SE, Montgomery HE. Review article: pancreatic renin-angiotensin systems in health and disease. *Aliment Pharmacol Ther* 2011; **34**: 840–52.
53. Steckelings UM, Czarnetzki BM. The renin-angiotensin-system in the skin. Evidence for its presence and possible functional implications. *Exp Dermatol* 1995; **4**: 329–34.
54. Leung PS, Sernia C. The renin-angiotensin system and male reproduction: new functions for old hormones. *J Mol Endocrinol* 2003; **30**: 263–70.
55. Hagemann A, Nielsen AH, Poulsen K. The uteroplacental renin-angiotensin system: a review. *Exp Clin Endocrinol* 1994; **102**: 252–61.
56. Mukhopadhyay AK, Brunswig-Spickenheier B. Follicular maturation and atresia – possible role of intraovarian regulatory factors. *J Reprod Fertil Suppl* 1996; **50**: 105–12.
57. Steckelings UM, Wollschlager T, Peters J, Henz BM, Hermes B, Artuc M. Human skin: source of and target organ for angiotensin II. *Exp Dermatol* 2004; **13**: 148–54.
58. Yvan-Charvet L, Quignard-Boulange A. Role of adipose tissue renin-angiotensin system in metabolic and inflammatory diseases associated with obesity. *Kidney Int* 2011; **79**: 162–8.
59. Stegbauer J, Lee DH, Seubert S, *et al.* Role of the renin-angiotensin system in autoimmune inflammation of the central nervous system. *Proc Natl Acad Sci USA* 2009; **106**: 14942–7.
60. Stegbauer J, Coffman TM. New insights into angiotensin receptor actions: from blood pressure to aging. *Curr Opin Nephrol Hypertens* 2011; **20**: 84–8.
61. Lubel JS, Herath CB, Burrell LM, Angus PW. Liver disease and the renin-angiotensin system: recent discoveries and clinical implications. *J Gastroenterol Hepatol* 2008; **23**: 1327–38.
62. Khairallah PA, Page IH. Effect of adrenergic agents on responses of smooth muscle to angiotensin. *Am J Physiol* 1962; **202**: 841–4.
63. Khairallah PA, Page IH. Effects of bradykinin and angiotensin on smooth muscle. *Ann N Y Acad Sci* 1963; **104**: 212–21.
64. Robertson PA, Rubin D. Stimulation of intestinal nervous elements by angiotensin. *Br J Pharmacol Chemother* 1962; **19**: 5–12.
65. Bruneval P, Hinglais N, Alhenc-Gelas F, *et al.* Angiotensin I converting enzyme in human intestine and kidney. Ultrastructural immunohistochemical localization. *Histochemistry* 1986; **85**: 73–80.
66. Hamming I, Timens W, Bulthuis ML, Lely AT, Navis GJ, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J Pathol* 2004; **203**: 631–7.
67. Harmer D, Gilbert M, Borman R, Clark KL. Quantitative mRNA expression profiling of ACE 2, a novel homologue of angiotensin converting enzyme. *FEBS Lett* 2002; **532**: 107–10.
68. Tipnis SR, Hooper NM, Hyde R, Karran E, Christie G, Turner AJ. A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. *J Biol Chem* 2000; **275**: 33238–43.
69. Kubota E, Dean RG, Hubner RA, Casley DJ, Johnston CI, Burrell LM. Differential tissue and enzyme inhibitory effects of the vasopeptidase inhibitor omapatrilat in the rat. *Clin Sci (Lond)* 2003; **105**: 339–45.
70. Shorning BY, Jarde T, McCarthy A, *et al.* Intestinal renin-angiotensin system is stimulated after deletion of Lkb1. *Gut* 2012; **61**: 202–13.
71. Ewert S, Spak E, Olbers T, Johnsson E, Edebo A, Fandriks L. Angiotensin II induced contraction of rat and human small intestinal wall musculature *in vitro*. *Acta Physiol (Oxf)* 2006; **188**: 33–40.
72. Spak E, Casselbrant A, Olbers T, Lonroth H, Fandriks L. Angiotensin II-induced contractions in human jejunal wall musculature *in vitro*. *Acta Physiol (Oxf)* 2008; **193**: 181–90.
73. Duggan KA, Mendelsohn FA, Levens NR. Angiotensin receptors and angiotensin I-converting enzyme in rat intestine. *Am J Physiol*. 1989; **257**: G504–10.
74. Sechi LA, Valentin JP, Griffin CA, Schambelan M. Autoradiographic characterization of angiotensin II receptor subtypes in rat intestine. *Am J Physiol* 1993; **265**: G21–7.
75. Smith RD. Characterisation of AT1 angiotensin receptors on cultured rat intestinal epithelial (RIE-1) cells. *Biochem Mol Biol Int* 1994; **32**: 923–31.
76. Seo MS, Fukamizu A, Saito T, Murakami K. Identification of a previously unrecognized production site of human renin. *Biochim Biophys Acta* 1991; **1129**: 87–9.
77. Wong TP, Debnam ES, Leung PS. Involvement of an enterocyte renin-angiotensin system in the local control of SGLT1-dependent glucose uptake across the rat small intestinal brush border membrane. *J Physiol*. 2007; **584**: 613–23.
78. Campbell DJ, Habener JF. Angiotensinogen gene is expressed and differentially regulated in multiple tissues of the rat. *J Clin Invest* 1986; **78**: 31–9.
79. Nagata S, Kato J, Kuwasako K, Kitamura K. Plasma and tissue levels of proangiotensin-12 and components of the renin-angiotensin system (RAS) following low- or high-salt feeding in rats. *Peptides* 2010; **31**: 889–92.
80. Johansson B, Holm M, Ewert S, Casselbrant A, Pettersson A, Fandriks L. Angiotensin II type 2 receptor-mediated duodenal mucosal alkaline secretion in the rat. *Am J Physiol Gastrointest Liver Physiol* 2001; **280**: G1254–60.
81. Levens NR, Peach MJ, Carey RM. Interactions between angiotensin peptides and the sympathetic nervous system mediating intestinal sodium and water absorption in the rat. *J Clin Invest* 1981; **67**: 1197–207.

© 2012 Blackwell Publishing Ltd

82. Levens NR. Response of isolated rat jejunum to angiotensin peptides. *Am J Physiol* 1986; **251**: G559–66.
83. Levens NR. Response of rat jejunum to angiotensin III: pharmacology and mechanism of action. *Am J Physiol* 1983; **245**: G511–8.
84. Jin XH, Wang ZQ, Siragy HM, Guerrant RL, Carey RM. Regulation of jejunal sodium and water absorption by angiotensin subtype receptors. *Am J Physiol* 1998; **275**: R515–23.
85. Carey RM, Levens NR, Peach MJ. Regulation of intestinal fluid transport by angiotensin II: mechanisms and physiological significance. *Trans Am Clin Climatol Assoc* 1984; **95**: 93–104.
86. Jin XH, Siragy HM, Guerrant RL, Carey RM. Compartmentalization of extracellular cGMP determines absorptive or secretory responses in the rat jejunum. *J Clin Invest* 1999; **103**: 167–74.
87. Erickson RH, Suzuki Y, Sedlmayer A, Song IS, Kim YS. Rat intestinal angiotensin-converting enzyme: purification, properties, expression, and function. *Am J Physiol* 1992; **263**: G466–73.
88. Bai JP. Distribution of brush-border membrane peptidases along the rat intestine. *Pharm Res* 1994; **11**: 897–900.
89. Kowalczuk S, Broer A, Tietze N, Vanslambrouck JM, Rasko JE, Broer S. A protein complex in the brush-border membrane explains a Hartnup disorder allele. *FASEB J* 2008; **22**: 2880–7.
90. Hirasawa K, Sato Y, Hosoda Y, Yamamoto T, Hanai H. Immunohistochemical localization of angiotensin II receptor and local renin-angiotensin system in human colonic mucosa. *J Histochem Cytochem* 2002; **50**: 275–82.
91. de los Rios AD, Labajos M, Manteca A, Morell M, Souviron A. Stimulatory action of angiotensin II on water and electrolyte transport by the proximal colon of the rat. *J Endocrinol* 1980; **86**: 35–43.
92. Fishlock DJ, Gunn A. The action of angiotensin on the human colon *in vitro*. *Br J Pharmacol* 1970; **39**: 34–9.
93. Jaszewski R, Tolia V, Ehrinpreis MN, *et al.* Increased colonic mucosal angiotensin I and II concentrations in Crohn's colitis. *Gastroenterology* 1990; **98**: 1543–8.
94. Hallersund P, Elfvin A, Helander HF, Fandriks L. The expression of renin-angiotensin system components in the human gastric mucosa. *J Renin Angiotensin Aldosterone Syst* 2011; **12**: 54–64.
95. Carl-McGrath S, Grantzdorffer I, Lendeckel U, Ebert MP, Rocken C. Angiotensin II-generating enzymes, angiotensin-converting enzyme (ACE) and mast cell chymase (CMA1), in gastric inflammation may be regulated by *H. pylori* and associated cytokines. *Pathology* 2009; **41**: 419–27.
96. Ludtke FE, Golenhofen K, Schubert F. Angiotensin II stimulates human gastric smooth muscle *in vitro*. *J Auton Pharmacol* 1989; **9**: 139–47.
97. Seno K, Zhu JH, Barrett JD, *et al.* Cigarette smoke increases gastric ulcer size in part by an angiotensin II-mediated mechanism in rats. *Dig Dis Sci* 1997; **42**: 74–8.
98. Cullen JJ, Ephgrave KS, Broadhurst KA, Booth B. Captopril decreases stress ulceration without affecting gastric perfusion during canine hemorrhagic shock. *J Trauma* 1994; **37**: 43–9.
99. Casselbrant A, Edebo A, Hallersund P, *et al.* Angiotensin II receptors are expressed and functional in human esophageal mucosa. *Am J Physiol Gastrointest Liver Physiol* 2009; **297**: G1019–27.
100. Casselbrant A, Edebo A, Wennerblom J, *et al.* Actions by angiotensin II on esophageal contractility in humans. *Gastroenterology* 2007; **132**: 249–60.
101. Matsuda T, Suzuki J, Furuya K, Masutani M, Kawakami Y. Serum angiotensin I-converting enzyme is reduced in Crohn's disease and ulcerative colitis irrespective of genotype. *Am J Gastroenterol* 2001; **96**: 2705–10.
102. Silverstein E, Fierst SM, Simon MR, Weinstock JV, Friedland J. Angiotensin-converting enzyme in Crohn's disease and ulcerative colitis. *Am J Clin Pathol* 1981; **75**: 175–8.
103. Sommer H, Schweisfurth H, Schulz M. Serum angiotensin-I-converting enzyme and carboxypeptidase N in Crohn's disease and ulcerative colitis. *Enzyme* 1986; **35**: 181–8.
104. Takeuchi N, Fukushima T, Sugita A, Tsuchiya S. [Angiotensin-converting-enzyme (ACE) in Crohn's disease]. *Nippon Shokakibyo Gakkai Zasshi* 1992; **89**: 596–600.
105. Letizia C, D'Ambrosio C, Agostini D, *et al.* Serum angiotensin converting enzyme activity in Crohn's disease and ulcerative colitis. *Ital J Gastroenterol* 1993; **25**: 23–5.
106. Studdy P, Bird R, James DG. Serum angiotensin-converting enzyme (SACE) in sarcoidosis and other granulomatous disorders. *Lancet* 1978; **2**: 1331–4.
107. Nunez-Gornes JF, Tewksbury DA. Serum angiotensin-converting-enzyme in Crohn's disease. *Am J Gastroenterol* 1981; **75**: 384–5.
108. Costerousse O, Danilov S, Alhenc-Gelas F. Genetics of angiotensin I-converting enzyme. *Clin Exp Hypertens* 1997; **19**: 659–69.
109. Saibeni S, Spina L, Virgilio T, *et al.* Angiotensin-converting enzyme insertion/deletion gene polymorphism in inflammatory bowel diseases. *Eur J Gastroenterol Hepatol* 2007; **19**: 976–81.
110. Papapetropoulos A, Antonov A, Virmani R, *et al.* Monocyte- and cytokine-induced downregulation of angiotensin-converting enzyme in cultured human and porcine endothelial cells. *Circ Res* 1996; **79**: 512–23.
111. Hume GE, Fowler EV, Lincoln D, *et al.* Angiotensinogen and transforming growth factor beta1: novel genes in the pathogenesis of Crohn's disease. *J Med Genet* 2006; **43**: e51.
112. Katada K, Yoshida N, Suzuki T, *et al.* Dextran sulfate sodium-induced acute colonic inflammation in angiotensin II type 1a receptor deficient mice. *Inflamm Res* 2008; **57**: 84–91.
113. Spencer AU, Yang H, Haxhija EQ, Wildhaber BE, Greenson JK, Teitelbaum DH. Reduced severity of a mouse colitis model with angiotensin converting enzyme inhibition. *Dig Dis Sci* 2007; **52**: 1060–70.
114. Koga H, Yang H, Adler J, Zimmermann EM, Teitelbaum DH. Transanal delivery of angiotensin converting enzyme inhibitor prevents colonic fibrosis in a mouse colitis model: development of a unique mode of treatment. *Surgery* 2008; **144**: 259–68.
115. Santiago OI, Rivera E, Ferder L, Appleyard CB. An angiotensin II receptor antagonist reduces inflammatory parameters in two models of colitis. *Regul Pept* 2008; **146**: 250–9.
116. Inokuchi Y, Morohashi T, Kawana I, Nagashima Y, Kihara M, Umemura S. Amelioration of 2,4,6-trinitrobenzene sulphonic acid induced colitis in angiotensinogen gene knockout mice. *Gut* 2005; **54**: 349–56.
117. Wengrower D, Zanninelli G, Pappo O, *et al.* Prevention of fibrosis in experimental colitis by captopril: the role of tgf-beta1. *Inflamm Bowel Dis* 2004; **10**: 536–45.

© 2012 Blackwell Publishing Ltd

118. Sturiale S, Barbara G, Qiu B, *et al.* Neutral endopeptidase (EC 3.4.24.11) terminates colitis by degrading substance P. *Proc Natl Acad Sci USA* 1999; **96**: 11653–8.
119. Platten M, Youssef S, Hur EM, *et al.* Blocking angiotensin-converting enzyme induces potent regulatory T cells and modulates TH1- and TH17-mediated autoimmunity. *Proc Natl Acad Sci USA* 2009; **106**: 14948–53.
120. Lanz TV, Ding Z, Ho PP, *et al.* Angiotensin II sustains brain inflammation in mice via TGF-beta. *J Clin Invest* 2010; **120**: 2782–94.
121. Crowley SD, Vasievich MP, Ruiz P, *et al.* Glomerular type 1 angiotensin receptors augment kidney injury and inflammation in murine autoimmune nephritis. *J Clin Invest* 2009; **119**: 943–53.
122. Lever AF, Hole DJ, Gillis CR, *et al.* Do inhibitors of angiotensin-I-converting enzyme protect against risk of cancer? *Lancet* 1998; **352**: 179–84.
123. Ronquist G, Rodriguez LA, Ruigomez A, *et al.* Association between captopril, other antihypertensive drugs and risk of prostate cancer. *Prostate* 2004; **58**: 50–6.
124. Sipahi I, Debanne SM, Rowland DY, Simon DI, Fang JC. Angiotensin-receptor blockade and risk of cancer: meta-analysis of randomised controlled trials. *Lancet Oncol* 2010; **11**: 627–36.
125. Pasternak B, Svanstrom H, Callreus T, Melbye M, Hviid A. Use of angiotensin receptor blockers and the risk of cancer. *Circulation* 2011; **123**: 1729–36.
126. Connolly S, Yusuf S, Swedberg K, *et al.* Effects of telmisartan, irbesartan, valsartan, candesartan, and losartan on cancers in 15 trials enrolling 138,769 individuals. *J Hypertens* 2011; **29**: 623–35.
127. George AJ, Thomas WG, Hannan RD. The renin-angiotensin system and cancer: old dog, new tricks. *Nat Rev Cancer* 2010; **10**: 745–59.
128. Kinoshita J, Fushida S, Harada S, *et al.* Local angiotensin II-generation in human gastric cancer: correlation with tumor progression through the activation of ERK1/2, NF-kappaB and survivin. *Int J Oncol* 2009; **34**: 1573–82.
129. Rocken C, Rohl FW, Diebler E, *et al.* The angiotensin II/angiotensin II receptor system correlates with nodal spread in intestinal type gastric cancer. *Cancer Epidemiol Biomarkers Prev* 2007; **16**: 1206–12.
130. Suganuma T, Ino K, Shibata K, *et al.* Functional expression of the angiotensin II type 1 receptor in human ovarian carcinoma cells and its blockade therapy resulting in suppression of tumor invasion, angiogenesis, and peritoneal dissemination. *Clin Cancer Res* 2005; **11**: 2686–94.
131. Anandanadesan R, Gong Q, Chipitsyna G, Witkiewicz A, Yeo CJ, Arafat HA. Angiotensin II induces vascular endothelial growth factor in pancreatic cancer cells through an angiotensin II type 1 receptor and ERK1/2 signaling. *J Gastrointest Surg* 2008; **12**: 57–66.
132. Kosugi M, Miyajima A, Kikuchi E, Horiguchi Y, Murai M. Angiotensin II type 1 receptor antagonist candesartan as an angiogenic inhibitor in a xenograft model of bladder cancer. *Clin Cancer Res* 2006; **12**: 2888–93.
133. Neo JH, Malcontenti-Wilson C, Muralidharan V, Christophi C. Effect of ACE inhibitors and angiotensin II receptor antagonists in a mouse model of colorectal cancer liver metastases. *J Gastroenterol Hepatol* 2007; **22**: 577–84.
134. Heinzerling JH, Anthony T, Livingston EH, Huerta S. Predictors of distant metastasis and mortality in patients with stage II colorectal cancer. *Am Surg* 2007; **73**: 230–8.
135. Sjoberg T, Garcia Rodriguez LA, Lindblad M. Angiotensin-converting enzyme inhibitors and risk of esophageal and gastric cancer: a nested case-control study. *Clin Gastroenterol Hepatol* 2007; **5**: 1160–6.
136. Fandriks L. The angiotensin II type 2 receptor and the gastrointestinal tract. *J Renin Angiotensin Aldosterone Syst* 2010; **11**: 43–8.
137. Aneman A, Bengtsson J, Snygg J, Holm M, Pettersson A, Fandriks L. Differentiation of the peptidergic vasoregulatory response to standardized splanchnic hypoperfusion by acute hypovolaemia or sepsis in anaesthetized pigs. *Acta Physiol Scand* 1999; **166**: 293–300.
138. Snygg J, Aneman A, Pettersson A, Fandriks L. Intestinal nitric oxide output during reduced mucosal blood flow in healthy volunteers. *Crit Care Med* 2003; **31**: 2198–204.
139. Laesser M, Fandriks L, Pettersson A, Ewert S, Aneman A. Angiotensin II blockade in existing hypovolemia: effects of candesartan in the porcine splanchnic and renal circulation. *Shock* 2000; **14**: 471–7.
140. Aneman A, Svensson M, Broome M, Biber B, Petterson A, Fandriks L. Specific angiotensin II receptor blockage improves intestinal perfusion during graded hypovolemia in pigs. *Crit Care Med* 2000; **28**: 818–23.
141. Oldner A, Wanecek M, Weitzberg E, *et al.* Angiotensin II receptor antagonism increases gut oxygen delivery but fails to improve intestinal mucosal acidosis in porcine endotoxin shock. *Shock* 1999; **11**: 127–35.
142. Tadros T, Traber DL, Heggers JP, Herndon DN. Angiotensin II inhibitor DuP753 attenuates burn- and endotoxin-induced gut ischemia, lipid peroxidation, mucosal permeability, and bacterial translocation. *Ann Surg* 2000; **231**: 566–76.
143. Luetscher JA, Kraemer FB, Wilson DM, Schwartz HC, Bryer-Ash M. Increased plasma inactive renin in diabetes mellitus. A marker of microvascular complications. *N Engl J Med* 1985; **312**: 1412–7.
144. Moriyama T, Fujibayashi M, Fujiwara Y, *et al.* Angiotensin II stimulates interleukin-6 release from cultured mouse mesangial cells. *J Am Soc Nephrol* 1995; **6**: 95–101.
145. Sayeski PP, Bernstein KE. Signal transduction mechanisms of the angiotensin II type AT-receptor: looking beyond the heterotrimeric G protein paradigm. *J Renin Angiotensin Aldosterone Syst* 2001; **2**: 4–10.
146. Xia Y, Ramin SM, Kellems RE. Potential roles of angiotensin receptor-activating autoantibody in the pathophysiology of preeclampsia. *Hypertension* 2007; **50**: 269–75.
147. Ruiz-Ortega M, Ruperez M, Lorenzo O, *et al.* Angiotensin II regulates the synthesis of proinflammatory cytokines and chemokines in the kidney. *Kidney Int Suppl* 2002; **62**: S12–22.
148. Willis LM, El-Remessy AB, Somanath PR, Deremer DL, Fagan SC. Angiotensin receptor blockers and angiogenesis: clinical and experimental evidence. *Clin Sci (Lond)* 2011; **120**: 307–19.
149. Santos RA, Ferreira AJ, Simoes ESAC. Recent advances in the angiotensin-converting enzyme 2-angiotensin(1–7)-Mas axis. *Exp Physiol* 2008; **93**: 519–27.
150. Ruiz-Ortega M, Lorenzo O, Egido J. Angiotensin III increases MCP-1 and activates NF-kappaB and AP-1 in cultured mesangial and mononuclear cells. *Kidney Int* 2000; **57**: 2285–98.
151. Yatabe J, Yoneda M, Yatabe MS, *et al.* Angiotensin III stimulates aldosterone secretion from adrenal gland partially via angiotensin II type

© 2012 Blackwell Publishing Ltd

2 receptor but not angiotensin II type 1 receptor. *Endocrinology* 2011; **152**: 1582–8.
152. Horiuchi M, Hayashida W, Akishita M, *et al.* Stimulation of different subtypes of angiotensin II receptors, AT1 and AT2 receptors, regulates STAT activation by negative crosstalk. *Circ Res* 1999; **84**: 876–82.
153. Rompe F, Artuc M, Hallberg A, *et al.* Direct angiotensin II type 2 receptor stimulation acts anti-inflammatory through epoxyeicosatrienoic acid and inhibition of nuclear factor kappaB. *Hypertension* 2010; **55**: 924–31.
154. Herrera M, Garvin JL. Angiotensin II stimulates thick ascending limb NO production via AT receptors and Akt1-dependent nitric-oxide synthase 3 (NOS3) activation. *J Biol Chem* 2010; **285**: 14932–40.
155. AbdAlla S, Lother H, Abdel-tawab AM, Quitterer U. The angiotensin II AT2 receptor is an AT1 receptor antagonist. *J Biol Chem* 2001; **276**: 39721–6.
156. Takai S, Jin D, Miyazaki M. New approaches to blockade of the renin-angiotensin-aldosterone system: chymase as an important target to prevent organ damage. *J Pharmacol Sci* 2010; **113**: 301–9.
157. Wan Y, Wallinder C, Plouffe B, *et al.* Design, synthesis, and biological evaluation of the first selective nonpeptide AT2 receptor agonist. *J Med Chem* 2004; **47**: 5995–6008.
158. Alterman M. Development of selective non-peptide angiotensin II type 2 receptor agonists. *J Renin Angiotensin Aldosterone Syst* 2010; **11**: 57–66.
159. Tsang SW, Ip SP, Leung PS. Prophylactic and therapeutic treatments with AT 1 and AT 2 receptor antagonists and their effects on changes in the severity of pancreatitis. *Int J Biochem Cell Biol* 2004; **36**: 330–9.
160. Faure S, Bureau A, Oudart N, Javellaud J, Fournier A, Achard JM. Protective effect of candesartan in experimental ischemic stroke in the rat mediated by AT2 and AT4 receptors. *J Hypertens* 2008; **26**: 2008–15.
161. Singh K, Singh T, Sharma PL. Beneficial effects of angiotensin (1–7) in diabetic rats with cardiomyopathy. *Ther Adv Cardiovasc Dis* 2011; **5**: 159–67.
162. Ferreira AJ, Moraes PL, Foureaux G, Andrade AB, Santos RA, Almeida AP. The angiotensin-(1–7)/mas receptor axis is expressed in sinoatrial node cells of rats. *J Histochem Cytochem* 2011; **59**: 761–8.
163. Zeng WT, Chen WY, Leng XY, *et al.* Impairment of cardiac function and remodeling induced by myocardial infarction in rats are attenuated by the nonpeptide angiotensin-(1–7) analog ave 0991. *Cardiovasc Ther* 2010; [Epub ahead of print].
164. da Silveira KD, Coelho FM, Vieira AT, *et al.* Anti-inflammatory effects of the activation of the angiotensin-(1–7) receptor, MAS, in experimental models of arthritis. *J Immunol* 2010; **185**: 5569–76.
165. He JG, Chen SL, Huang YY, Chen YL, Dong YG, Ma H. The nonpeptide AVE0991 attenuates myocardial hypertrophy as induced by angiotensin II through downregulation of transforming growth factor-beta1/Smad2 expression. *Heart Vessels* 2010; **25**: 438–43.
166. Toton-Zuranska J, Gajda M, Pyka-Fosciak G, *et al.* AVE 0991-angiotensin-(1–7) receptor agonist, inhibits atherogenesis in apoE-knockout mice. *J Physiol Pharmacol* 2010; **61**: 181–3.
167. Zhong J, Basu R, Guo D, *et al.* Angiotensin-converting enzyme 2 suppresses pathological hypertrophy, myocardial fibrosis, and cardiac dysfunction. *Circulation*. 2010; **122**: 717–28.
168. Oudit GY, Penninger JM. Recombinant human angiotensin-converting enzyme 2 as a new renin-angiotensin system peptidase for heart failure therapy. *Curr Heart Fail Rep* 2011; **8**: 176–83.
169. Kim MA, Yang D, Kida K, *et al.* Effects of ACE2 inhibition in the post-myocardial infarction heart. *J Card Fail* 2010; **16**: 777–85.
170. Rouleau JL, Pfeffer MA, Stewart DJ, *et al.* Comparison of vasopeptidase inhibitor, omapatrilat, and lisinopril on exercise tolerance and morbidity in patients with heart failure: IMPRESS randomised trial. *Lancet* 2000; **356**: 615–20.
171. Packer M, Califf RM, Konstam MA, *et al.* Comparison of omapatrilat and enalapril in patients with chronic heart failure: the Omapatrilat Versus Enalapril Randomized Trial of Utility in Reducing Events (OVERTURE). *Circulation* 2002; **106**: 920–6.
172. Kostis JB, Packer M, Black HR, Schmieder R, Henry D, Levy E. Omapatrilat and enalapril in patients with hypertension: the Omapatrilat Cardiovascular Treatment vs. Enalapril (OCTAVE) trial. *Am J Hypertens* 2004; **17**: 103–11.
173. Ruilope LM, Dukat A, Bohm M, Lacourciere Y, Gong J, Lefkowitz MP. Blood-pressure reduction with LCZ696, a novel dual-acting inhibitor of the angiotensin II receptor and neprilysin: a randomised, double-blind, placebo-controlled, active comparator study. *Lancet* 2010; **375**: 1255–66.
174. Ichihara A, Hayashi M, Kaneshiro Y, *et al.* Inhibition of diabetic nephropathy by a decoy peptide corresponding to the "handle" region for nonproteolytic activation of prorenin. *J Clin Invest* 2004; **114**: 1128–35.
175. Ichihara A, Sakoda M, Kurauchi-Mito A, Nishiyama A, Itoh H. Involvement of receptor-bound prorenin in development of nephropathy in diabetic db/db mice. *J Am Soc Hypertens* 2008; **2**: 332–40.
176. Ichihara A, Suzuki F, Nakagawa T, *et al.* Prorenin receptor blockade inhibits development of glomerulosclerosis in diabetic angiotensin II type 1a receptor-deficient mice. *J Am Soc Nephrol* 2006; **17**: 1950–61.
177. Ichihara A, Kaneshiro Y, Suzuki F. Prorenin receptor blockers: effects on cardiovascular complications of diabetes and hypertension. *Expert Opin Investig Drugs* 2006; **15**: 1137–9.
178. Satofuka S, Ichihara A, Nagai N, Tsubota K, Itoh H, Ishida S. Pathologic roles of prorenin and (pro)renin receptor in the eye. *Front Biosci* 2008; **13**: 3884–95.
179. Satofuka S, Ichihara A, Nagai N, *et al.* Role of nonproteolytically activated prorenin in pathologic, but not physiologic, retinal neovascularization. *Invest Ophthalmol Vis Sci* 2007; **48**: 422–9.
180. Satofuka S, Ichihara A, Nagai N, *et al.* Suppression of ocular inflammation in endotoxin-induced uveitis by inhibiting nonproteolytic activation of prorenin. *Invest Ophthalmol Vis Sci* 2006; **47**: 2686–92.

© 2012 Blackwell Publishing Ltd