

Modulation of the action of insulin by angiotensin-(1–7)

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Abstract

The prevalence of Type 2 diabetes mellitus is predicted to increase dramatically over the coming years and the clinical implications and healthcare costs from this disease are overwhelming. In many cases, this pathological condition is linked to a cluster of metabolic disorders, such as obesity, systemic hypertension and dyslipidaemia, defined as the metabolic syndrome. Insulin resistance has been proposed as the key mediator of all of these features and contributes to the associated high cardiovascular morbidity and mortality. Although the molecular mechanisms behind insulin resistance are not completely understood, a negative cross-talk between AngII (angiotensin II) and the insulin signalling pathway has been the focus of great interest in the last decade. Indeed, substantial evidence has shown that anti-hypertensive drugs that block the RAS (renin–angiotensin system) may also act to prevent diabetes. Despite its long history, new components within the RAS continue to be discovered. Among them, Ang-(1–7) [angiotensin-(1–7)] has gained special attention as a counter-regulatory hormone opposing many of the AngII-related deleterious effects. Specifically, we and others have demonstrated that Ang-(1–7) improves the action of insulin and opposes the negative effect that AngII exerts at this level. In the present review, we provide evidence showing that insulin and Ang-(1–7) share a common intracellular signalling pathway. We also address the molecular mechanisms behind the beneficial effects of Ang-(1–7) on AngII-mediated insulin resistance. Finally, we discuss potential therapeutic approaches leading to modulation of the ACE2 (angiotensin-converting enzyme 2)/Ang-(1–7)/Mas receptor axis as a very attractive strategy in the therapy of the metabolic syndrome and diabetes-associated diseases.

Key words: angiotensin-(1–7), angiotensin-converting enzyme 2 (ACE2), insulin, Mas receptor, renin–angiotensin system (RAS)

INTRODUCTION

Diabetes mellitus is emerging as a worldwide epidemic, and the clinical implications and healthcare costs from the disease are overwhelming [1]. In many cases, this pathological condition is linked to obesity, systemic hypertension and hyperlipidaemia forming the so-called metabolic syndrome [2–4]. Insulin resistance is believed to be the pathogenic mediator of all these features and contributes to the associated high cardiovascular morbidity and mortality [3,5,6]. Resistance of the action of insulin is a state in which insulin-sensitive tissues (i.e. adipose tissue, liver and skeletal muscle) exhibit a failure to respond to normal circulating levels of insulin. To compensate for this inad-

equately response, pancreatic β -cells augment insulin production leading to hyperinsulinaemia [7]. Insulin resistance is associated with impaired insulin-mediated inhibition of gluconeogenesis, reduced skeletal muscle uptake of glucose, hyperglycaemia, inhibition of lipolysis and increased plasma levels of non-esterified ('free') fatty acids. Long-term resistance to the action of insulin and its consequent hypersecretion of insulin, eventually lead to a pancreatic β -cell failure, causes a pre-diabetic state and glucose intolerance, which can later progress to Type 2 diabetes [3,5–7].

The molecular mechanisms behind insulin resistance are not completely understood. However, it is clear that the cross-talk between insulin and other hormones, such as AngII (angiotensin II) [8–13], cortisol [14], adrenaline (epinephrine) [15], growth

Abbreviations: ACE, angiotensin-converting enzyme; Ang-(1–7), angiotensin-(1–7); AngI, angiotensin I; AngII, angiotensin II; ARB, angiotensin receptor blocker; AS160, Akt substrate of 160 kDa; AT₁R, AngII type 1 receptor; AT₂R, AngII type 2 receptor; EGF, epidermal growth factor; eNOS, endothelial NOS; ERK, extracellular-signal-regulated kinase; FOXO1, forkhead box O1; GLUT4, glucose transporter 4; GSK-3, glycogen synthase kinase-3; IKK, inhibitor of nuclear factor κ B kinase; IR, insulin receptor; IRS, IR substrate; JAK2, Janus kinase 2; JNK, c-Jun N-terminal kinase; KO, knockout; LV, left ventricular; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; mTORC, mTOR complex; NOS, nitric oxide synthase; PDK1, phosphoinositide-dependent kinase; PI3K, phosphoinositide 3-kinase; PKB, protein kinase B; PKC, protein kinase C; PIP₃, phosphatidylinositol 3,4,5-trisphosphate; PPAR γ , peroxisome-proliferator-activated receptor γ ; RAS, renin–angiotensin system; ROS, reactive oxygen species; SH2, Src homology 2; Shc, Src homology and collagen homology; STAT, signal transducer and activator of transcription; STZ, streptozotocin; VSMC, vascular smooth muscle cell.

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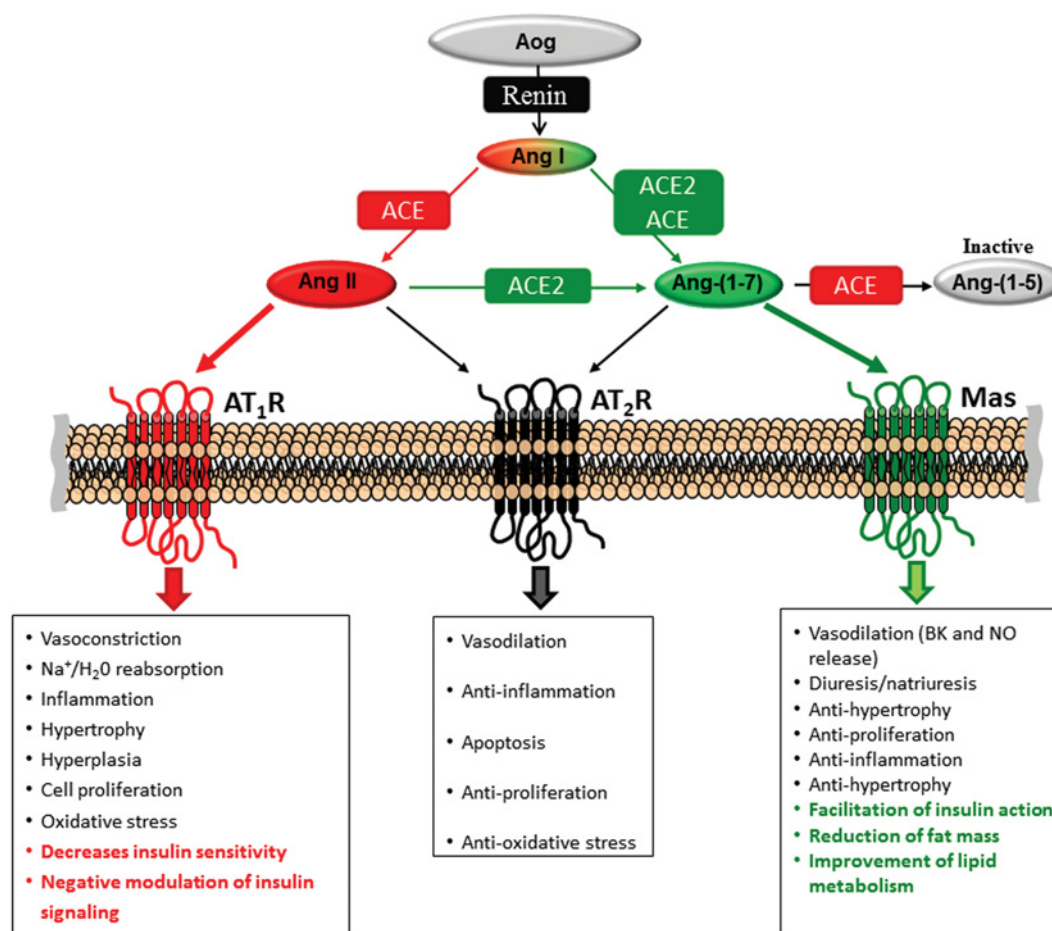


Figure 1 Schematic diagram of the RAS

Red: the pathway leading to AngII generation and its deleterious effect on insulin signaling via the AT₁R. Green: the pathway leading to Ang-(1-7) synthesis and its positive modulation of insulin actions via the Mas receptor. Aog, angiotensinogen; BK, bradykinin.

hormone [16], leptin [17] and adiponectin [18], can modulate the final outcome of the action of insulin. In particular, the participation of the RAS (renin-angiotensin system) in the modulation of the action of insulin has been the focus of great interest. Indeed, several studies demonstrating a close connection between insulin resistance and cardiovascular disease support the notion that alterations within the RAS could be related to dysregulation of the action of insulin [19–21]. Specifically, it has been demonstrated that AngII plays a critical role in the aetiology of insulin resistance [22,23]. The mechanism behind this deleterious effect appears to be related to a negative modulation exerted by AngII on several steps of the insulin-signalling cascade, including insulin-induced phosphorylation of the IR (insulin receptor), IRS-1 (IR substrate-1) and activation of Akt [also known as PKB (protein kinase B)] by PI3K (phosphoinositide 3-kinase) [8–13,24,25]. Accordingly, recent long-term large-scale clinical trials have shown that inhibition of ACE (angiotensin-converting enzyme) or selective blockade of the AT₁R (AngII type 1 receptor) improves glycaemic control in patients with diabetes and prevents new-onset of diabetes mellitus in patients without diabetes [26–28]. In line with these reports, several studies have shown that animal models of

insulin resistance and/or Type 2 diabetes display enhanced insulin sensitivity [29–33] and an enhancement in the response to insulin at various steps in the insulin-signalling cascade [34,35] as a consequence of lower AngII formation or inhibition of its actions. These data clearly indicate that the signalling cross-talk between insulin and AngII has significant physiological relevance.

Despite its long history, new components and interactions between novel and established components of the RAS continue to be discovered. The RAS is classically conceived as a major regulator of body fluid and cardiovascular homeostasis. It consists primarily of an enzymatic cascade through which angiotensin is sequentially converted into AngI (angiotensin I) and later the generation of AngII through the action of renin and the ACE respectively (Figure 1). AngII mediates its specific functions via type 1 and type 2 receptors, i.e. AT₁R and AT₂R (AngII type 2 receptor) (Figure 1). Most of the effects of AngII are mediated by the AT₁R, including vasoconstriction, and pro-inflammatory, pro-oxidative, proliferative and hypertrophic effects (Figure 1); the actions mediated by the AT₂R, in general, oppose those mediated by the AT₁R (Figure 1). By the action of ACE2, a homologue of ACE, Ang-(1-7) [angiotensin-(1-7)] is generated directly from

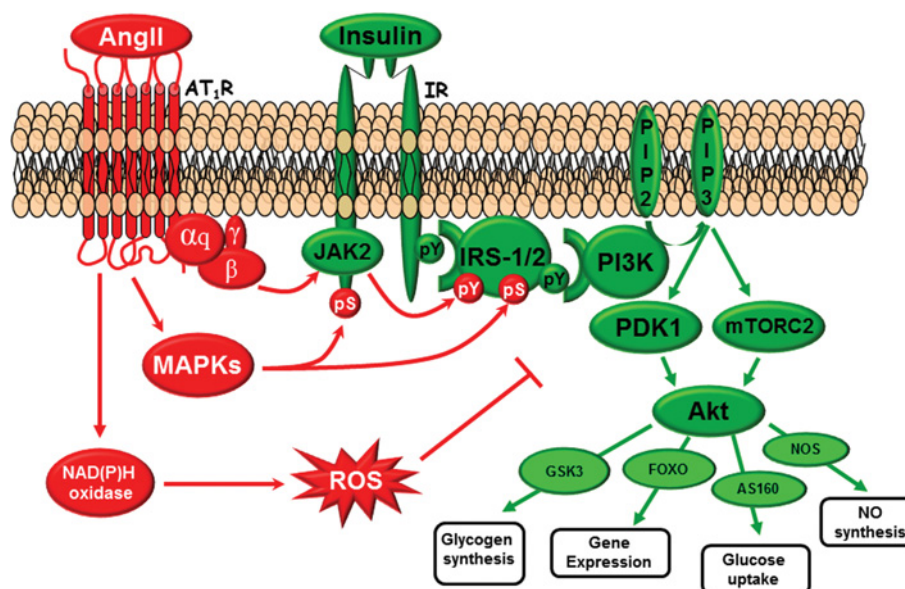


Figure 2 Negative cross-talk between insulin and the AngII signalling pathway

Proteins activated by insulin are shown in green and proteins activated by AngII are shown in red.

AngII and also indirectly from AngI (Figure 1). Ang-(1-7) binds to and activates the GPCR (G-protein-coupled receptor) Mas, through which Ang-(1-7) induces responses in opposition to those of AngII, including vasodilation, anti-hypertrophic effects and anti-proliferative properties [36–39] (Figure 1). Importantly, Ang-(1-7) seems to contribute to the anti-hypertensive effects of the blockade of the RAS [40–42]. Thus it is clear that the balance between the ACE/AngII/AT₁R axis and the ACE2/Ang-(1-7)/Mas receptor axis has great importance in the control of several body functions apart from the regulation of cardio-renal actions [39] (Figure 1). Given the major physiological relevance of this area, research on the ACE2/Ang-(1-7)/Mas receptor axis has expanded greatly over the last few years providing evidence for a metabolic role of the axis and supporting the concept that Ang-(1-7) participates in the maintenance of normoglycaemia. The circulating concentrations of Ang-(1-7) are within the picomolar range [43] and, interestingly, patients with Type 2 diabetes exhibit decreased circulating concentrations of Ang-(1-7) [44]. This is in good correlation with the observation that pregnancy tends to elevate the plasma level of Ang-(1-7), but women with gestational diabetes have lower levels of Ang-(1-7) compared with healthy pregnant women [45]. In general it appears that local levels of Ang-(1-7) correlate with ACE2 levels [46,47]. Administration of a high-fat diet to mice is associated with insulin resistance and results in reduced kidney ACE2 activity, increased levels of plasma AngII and decreased levels of plasma Ang-(1-7) [48].

In the present review, we will discuss recent evidence suggesting that the improvement in insulin resistance associated with different therapies might be mediated, at least in part, by Ang-(1-7) as a positive modulator of the action of insulin (Figure 1). We will focus on the negative cross-talk between the insulin-signalling pathway and AngII and the beneficial effects exerted by Ang-(1-7) at this level. Finally, we will discuss potential therapeutic

applications of the ACE2/Ang-(1-7)/Mas receptor axis for the treatment of various alterations associated with Type 2 diabetes.

THE INSULIN SIGNALLING SYSTEM: BASIC CONCEPTS AND REGULATION

The IR is a tetrameric protein that consists of two α -subunits and two β -subunits. Insulin binding to the α -subunit leads to activation of the kinase activity residing in the β -subunit, followed by autophosphorylation of tyrosine residues in several regions of the cytoplasmic portion of the β -subunit and a further increase in its kinase activity [49]. The IR kinase phosphorylates the tyrosine residues of several cytosolic proteins [49,50]. Many insulin responses require the phosphorylation of IRS-1 and IRS-2 (Figure 2). Additional IR substrates include isoforms of the transforming protein Shc2 (Src homology and collagen homology 2), APS (adaptor protein with pleckstrin homology and src), c-Cbl (the proto-oncogene Casitas b-lineage lymphoma) and STAT5b (signal transducer and activator of transcription 5b) [50,51]. When tyrosine-phosphorylated, IRS proteins bind several SH2 (Src homology 2)-domain-containing proteins, which further propagate downstream signals. Some of these SH2-domain-containing proteins are adaptor proteins, such as the p85 regulatory subunit of PI3K and Grb2 (growth factor receptor-bound protein 2) [50]. Others have intrinsic enzymatic activity, such as the phosphotyrosine phosphatase SHP-2 (Src homology 2 domain-containing protein tyrosine phosphatase 2) and the cytoplasmic tyrosine kinases Fyn and JAK2 (Janus kinase 2) [50–52]. Substrate binding to these SH2 proteins can regulate their activities and/or subcellular location. Collectively, those molecules orchestrate the numerous insulin-mediated physiological responses.

A total of eight mammalian PI3Ks have been identified and grouped into three classes (I, II and III) according to their

sequence homology and *in vitro* substrate specificity [53]; the PI3Ks that are involved in the action of insulin belong to class I. PI3K is necessary for many, if not all, of the actions of insulin, including stimulation of glucose transport, activation of glycogen synthase and inhibition of hepatic gluconeogenesis [50]. Engagement of PI3K by the IRS proteins activates this lipid kinase at the plasma membrane, where its substrate, PIP₂ (phosphatidylinositol 4,5-bisphosphate), is abundant, stimulating the production of the key lipid second messenger PIP₃ (phosphatidylinositol 3,4,5-trisphosphate). PIP₃ then binds the PH (pleckstrin homology) domain of the serine/threonine kinase Akt, allowing two other kinases, PDK1 (phosphoinositide-dependent kinase 1) and mTORC2 [mTOR (mammalian target of rapamycin) complex 2], to phosphorylate and activate Akt/PKB [50] (Figure 2). Akt/PKB is a major effector of the insulin response, and its downstream substrates directly mediate many of the metabolic effects of insulin [54,55]. Well-established Akt substrates include GSK-3 (glycogen synthase kinase-3), which participates in the regulation of glycogen synthesis, the Rab GTPase-activating protein AS160 (Akt substrate of 160 kDa), involved in regulation of glucose transport, the small GTPase Rheb, involved in activation of TSC1–2 (tuberous sclerosis complex 1–2), regulation of mTOR and protein synthesis, FOXO1 (forkhead box O1) transcription factors, involved in regulation of expression of gluconeogenic and other genes, NOS (nitric oxide synthase), with a crucial role in the vasodilating effects of insulin, and BAD (Bcl-2/Bcl-xL-antagonist) causing cell death [54,55].

With regards to JAK2, insulin induces the tyrosine phosphorylation of this cytosolic tyrosine kinase and promotes its association with the IR, IRS proteins, members of the STAT family and Shc [52,56–62]. In response to insulin administration *in vivo*, JAK2 associates with the IR and becomes tyrosine-phosphorylated in insulin-sensitive tissues [61,62]. *In vitro*, tyrosine phosphorylation elicited by the IR or by JAK2 occurs at different sites in IRS-1 [52] (Figure 2). The physiological meaning of this phenomenon is not completely understood. Moreover, it has been reported that JAK2 participates in the insulin-stimulated mitogenic pathway, but not in its metabolic pathway [63]. However, in a state of insulin resistance, heightened JAK2 activation may attenuate insulin-induced Akt activation [63]. In this manner, JAK2 acts as an inhibitor of insulin signal transduction at the level of Akt, negatively affecting glucose metabolism.

Activation of the Ras/Raf/MEK [MAPK (mitogen-activated protein kinase)/ERK (extracellular-signal-regulated kinase) kinase]/ERK pathway is another major mechanism of insulin action and results in the activation of several MAPKs [64,65]. Various lines of observation indicate that both the IRS and the SHC family of proteins participate in this pathway. Moreover, in contrast with the final cellular events triggered by the PI3K/Akt signalling module, activation of MAPKs by insulin has a direct role in the growth-promoting effects of this hormone, and appears to be relatively dispensable in insulin-mediated metabolic regulation [50].

As stated above, tyrosine phosphorylation serves to propagate the signal emanating from the IR. However, in the last two decades, several mechanisms that control insulin signalling have been described. Among them, serine and threonine phosphoryla-

tion of the IR and its substrates (IRS proteins) has emerged as a key negative regulator of insulin signalling propagation [51,66] (Figure 2). There is evidence that serine phosphorylation of the IR impairs its autophosphorylation of tyrosine residues after insulin binding, and probably contributes to the impaired insulin signalling related to the metabolic syndrome [51,66]. Many protein kinases have been suggested as potential mediators of the serine/threonine phosphorylation of the IR. Cell-based and isolated protein studies have demonstrated that PKC (protein kinase C) mediates the phosphorylation of the IR at Ser⁹⁶⁷ and Ser⁹⁶⁸ of the juxtamembrane region [67], Ser⁹⁹⁴, Ser¹⁰³⁵ and Ser¹⁰³⁷ in the catalytic domain [68,69], and Ser¹²⁸⁸, Ser¹³⁰⁵, Ser¹³⁰⁶, Ser¹³²¹, Ser¹³²⁷ and Thr¹³⁴⁸ in the C-terminus [67,70,71]. It is not clear whether these phosphorylation sites are actually involved in the regulation of activation of the IR. However, increased PKC activity is thought to play a significant role in several models of human insulin resistance [72]. For instance, PKC β activity is increased in the muscle of obese insulin-resistant subjects [73], whereas inhibition of PKC activity can reverse the impaired insulin-mediated glucose uptake in muscle strips obtained from obese subjects [74]. Indeed, lipid infusion in rats increases serine phosphorylation of the IR and reduces insulin-stimulated tyrosine phosphorylation of the IR in a PKC-dependent manner [72,75]. JNK (c-Jun N-terminal kinase) and MAPKs have also been suggested as putative kinases involved in the serine phosphorylation of the IR. These enzymes are also activated by lipid accumulation and are associated with impaired insulin signalling [72]. In support of this, the decrease in autophosphorylation of the IR induced by lipid incubation in 3T3-L1 adipocytes is prevented by blocking JNK expression [76]. In addition, we have demonstrated that obese Zucker rats displayed an increased level of phosphorylation of the IR at Ser⁹⁹⁴ that was reduced after chronic treatment with the AT₁R blocker irbesartan [77]. These data propose that AngII-induced serine phosphorylation of the IR could be a mechanism behind the negative cross-talk between the insulin and AngII signalling pathways (Figure 2).

Unlike the scant information regarding serine/threonine phosphorylation of the IR, the influence of this kind of phosphorylation in the co-ordination of IRS function has been widely studied. A number of different mechanisms appear to be involved in the tyrosine dephosphorylation of the IRS proteins, their dissociation from the IR, and their intracellular localization and eventual degradation. Specifically, most of the information regarding the serine phosphorylation events taking place on the IRS proteins is available for IRS-1, whereas much less is known about the regulation of IRS-2 [51]. Potential inhibitory serine/threonine residues have been suggested near tyrosine residues in PI3K-binding motifs or proximal to the IRS-1 PTB (phosphotyrosine-binding domain) [78]. One of the major sites of regulatory phosphorylation within IRS-1 is Ser³⁰⁷ (human Ser³¹²) that was identified in CHO (Chinese-hamster ovary) cells treated with a JNK agonist [79]. Together with JNK, the serine/threonine kinase IKK β (inhibitor of nuclear factor κ B kinase β) has also been suggested as a potential kinase for Ser³⁰⁷ [80]. As proof of this, many factors implicated in the development of insulin resistance, such as TNF α (tumour necrosis factor α), non-esterified fatty acids and serine phosphatase inhibitors, are able to activate the

IKK complex and JNK [80,81]. Besides JNK and IKK β , to date several serine/threonine kinases have been reported to be involved in the phosphorylation of this particular site; many of them are activated by insulin itself. In cultured cells, insulin stimulates phosphorylation of IRS-1 at Ser³⁰⁷ via PI3K [82,83]. In addition, mTOR can mediate this insulin-stimulated Ser³⁰⁷ phosphorylation [84]. In line with these observations, we have shown that insulin-resistant rats exhibit enhanced phosphorylation of IRS-1 at Ser³⁰⁷ in adipose tissue and skeletal muscle in association with enhanced activation of mTOR and JNK [85]. In addition to Ser³⁰⁷, Ser⁶¹² has emerged as a potential site of phosphorylation and regulation of IRS-1 function, with ERK1/2 being the main kinases involved in the process [86]. Consistent with a pathological effect of ERK-dependent IRS-1 phosphorylation, Ser⁶³² (human Ser⁶³⁶) phosphorylation is elevated in muscle cells from individuals with Type 2 diabetes, and this is reduced by chemical inhibition of ERK1/2 activity [87].

SIGNALLING MOLECULES ENGAGED BY ANG-(1–7)

Our current knowledge of the signalling pathways that are regulated by Ang-(1–7) is limited and originates from both *in vivo* and *in vitro* studies that have primarily used Western blot analysis as a tool [88–92]. In addition, time-resolved quantitative phosphoproteomics using human endothelial cells has shed light on Ang-(1–7) signalling [93]. Both approaches have generated consistent results showing that insulin and Ang-(1–7) share signalling events, implying a cross-talk between these two signalling systems. Given that Ang-(1–7) can improve insulin sensitivity, this signalling overlap could be implicated in this metabolic role of Ang-(1–7). The main findings of these studies are discussed below.

Node JAK/STAT

The cytosolic tyrosine kinase JAK2 becomes activated and associates with the AT₁R in response to AngII [94,95]. JAK2 activation by AngII leads to the phosphorylation of STAT1 and STAT3 [96]. We have demonstrated that Ang-(1–7) stimulates the activity of JAK2 in the rat heart *in vivo*, and that this activation proceeds through a mechanism that involves the AT₁R since it is abolished by losartan and it is not prevented by either Mas or AT₂R antagonists [89]. In line with these findings, we have shown that Ang-(1–7) stimulates the phosphorylation of STAT3 and STAT5 in the rat heart by an AT₁R-mediated mechanism [90]. The pattern of activation of these signalling molecules was both time- and concentration-dependent, and similar to that initiated by AngII [89,90]. The participation of the JAK/STAT signalling pathway in the signalling pathways of Ang-(1–7) is intriguing. Activation of the JAK/STAT pathway is critical for the development of AngII-induced hypertension by mediating its effects on renal sodium excretory capability, but the physiological control of blood pressure by AngII appears not to require JAK2 activation [97,98]. Moreover, activation of the JAK/STAT cascade can stimulate excessive proliferation and growth of VSMCs

(vascular smooth muscle cells) and glomerular mesangial cells, contributing to the accelerated atherosclerosis and nephropathy observed in the diabetic state [99]. A potential explanation for this observation could be that, at the doses used, Ang-(1–7) could have activated JAK2 through the AT₁R or AT₂R, although it is not clear whether this could happen under physiological conditions. In any event, the participation of Ang-(1–7) as a stimulator of the JAK/STAT pathway contradicts its anti-atherogenic and anti-diabetic effects and deserves further exploration.

Node IRS proteins

Ang-(1–7) is capable of stimulating the phosphorylation of IRS-1 in the rat heart [89]. We have demonstrated that this stimulating effect of Ang-(1–7) is not mediated by the IR kinase, but instead involves the AT₁R through a mechanism that requires participation of JAK2 [89]. Both the time and concentration necessary to attain the maximum stimulatory response were similar to those described previously for AngII [100,101]. The demonstration that IRS-1 is used by Ang-(1–7) reinforces the previously postulated concept that the IRS proteins serve as convergence sites for the signal transduction of several hormones and cytokines [50,102,103]. Using systematic binding analysis in HEK (human embryonic kidney)-293 cells stably transfected with the AT₁R, it was shown that Ang-(1–7) exhibits minimal binding of the AT₁R (at 1 μ M) [104]. Although this study employed an artificial system, this confirms previous studies that have shown that Ang-(1–7) binds to the AT₁R with a very low affinity [105–107]; however, it should be noted that, in the literature, binding data are not always matched with functional data. There are several reports showing that some of the effects of Ang-(1–7) appear to be mediated by the AT₁R [108–112]. This apparent mismatch could be attributed to tissue heterogeneity in the AT₁R, AT₂R and Mas receptor and/or differential receptor subtype expression. In addition, interaction of the AT₁R with the Mas receptor could be relevant for Ang-(1–7) signal transduction and action. A functional interaction has been described between the AT₁R and the Mas receptor [113]. The Mas receptor can hetero-oligomerize with the AT₁R and, by so doing, inhibit the actions of AngII. [114]. Moreover, at high doses, Ang-(1–7) has been shown to down-regulate the AT₁R [115,116]. Thus our observation that Ang-(1–7) recruits JAK2, STAT3, STAT5 and IRS-1 in the rat heart through an AT₁R-mediated mechanism can be included among these atypical observations and suggests that Ang-(1–7) can use the AT₁R to transmit some of its signal to the inside of the cell.

Node PI3K/Akt

The serine/threonine kinase Akt appears to have a central role in Ang-(1–7) signalling. Phosphorylation of Akt at the regulatory sites Ser⁴⁷³ and Thr³⁰⁸ is stimulated by Ang-(1–7) in human endothelial cells *in vitro* [88], as well as in the rat heart [89], liver, skeletal muscle and adipose tissue [91] *in vivo* (Figure 3A). Indirect evidence for the participation of PI3K in Ang-(1–7)-induced Akt activation was provided through the use of the inhibitor of class I PI3Ks wortmannin [88,89]. Since then, the participation of the PI3K/Akt pathway in the action of Ang-(1–7) has been consistently reproduced in different experimental settings, particularly in cardiomyocytes [117]. Time-resolved quantitative

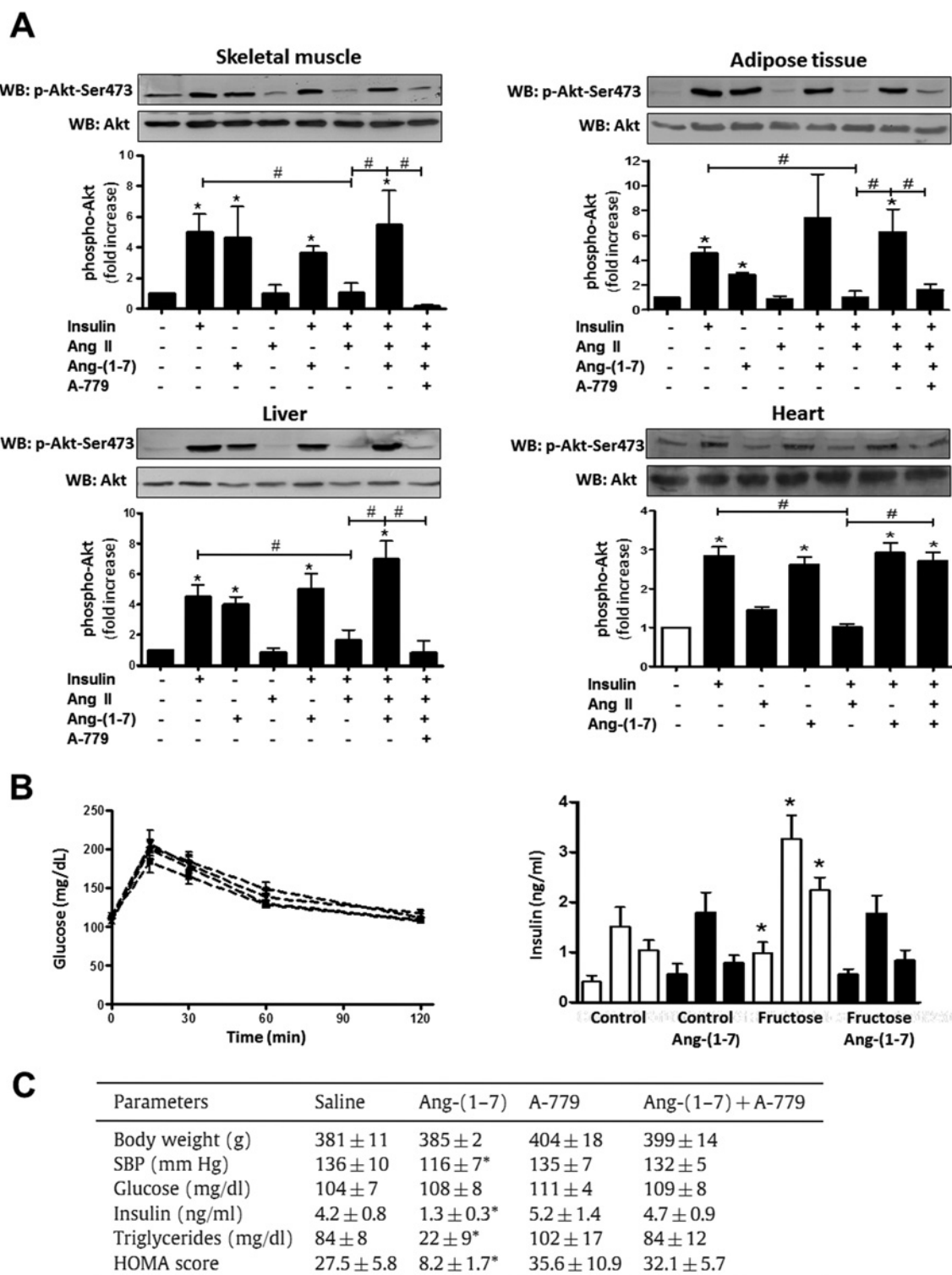


Figure 3 **Results from our laboratory demonstrating a positive modulation of insulin signalling by Ang-(1-7)**

(A) Acute *in vivo* administration of Ang-(1-7) induced the phosphorylation of Akt at Ser⁴⁷³ (p-Akt-Ser473) in the skeletal muscle, adipose tissue, liver and heart. Insulin-induced Akt was impaired when co-injected with AngII. This impairment was reversed by the presence of Ang-(1-7) and the Mas receptor-specific antagonist A-779 abolished this beneficial effect. **P* < 0.05 compared with the control group (saline-injected animals); #*P* < 0.05. WB, Western blot. (B) Ang-(1-7) reverses insulin resistance in fructose-fed rats. Control and fructose-fed rats (Fructose) were treated with Ang-(1-7) or saline. Although glucose tolerance was unaltered by the high-fructose diet, circulating insulin concentrations at the baseline and 15 min and 120 min after glucose administration were significantly higher in fructose-fed rats compared with the

phosphoproteomics analysis corroborated these findings, situating Akt in a central place in the action of Ang-(1–7) [93]. The participation of Ang-(1–7) as a modulator of lipid metabolism in adipose tissue has recently been evaluated and Ang-(1–7) was found to increase glycerol release from primary adipocytes in a dose-dependent manner [118]. This lipolytic effect of Ang-(1–7) appeared to be mediated by a Mas receptor/PI3K-dependent mechanism [118]. Interestingly, additional effects of Ang-(1–7) on adipose tissue have recently been reported, indicating that it promotes formation of small insulin-sensitive newly differentiated adipocytes [119]. This induction of adipogenesis by Ang-(1–7) proceeds via activation of PI3K/Akt [119]. Recently, it was shown that in patients with diabetes both the survival and proliferation of CD34⁺ cells is enhanced by Ang-(1–7) in a Mas/PI3K/Akt-dependent manner [120]. Additional reports have shown that engagement of Akt by Ang-(1–7) appears to be essential for other actions exerted by this hormone, such as the maintenance of cardiomyocyte function [117] and stimulation of atrial natriuretic peptide secretion [121].

Aside from class I PI3K, phosphoproteomics analysis identified PI3KC2A, a class II PI3K, as a target of Ang-(1–7) [93]. These enzymes are insensitive to PI3K inhibitors and have been shown to have a major role in the action of insulin by stimulating insulin secretion [122]. Interestingly, it was found previously that PI3KC2A contributes to maximal insulin-induced translocation of GLUT4 (glucose transporter 4) to the plasma membrane and subsequent glucose uptake [123]. Moreover, another report identified signal transduction via PI3KC2A as a novel pathway whereby insulin activates Akt and thus identifies PI3KC2A as a potential pharmacological target in Type 2 diabetes [124]. Thus the engagement of PI3KC2A by Ang-(1–7) is in good agreement with the insulin-sensitizing effect of this heptapeptide.

Akt has several cytosolic substrates, including NOS, GSK-3, AS160 and FOXO1 [54], and the information available so far suggests that all of these substrates are phosphorylated by Ang-(1–7). Ang-(1–7) activates eNOS (endothelial NOS) leading to vasodilation. This process is triggered by the Mas receptor [125] and requires activation of Akt [88,117]. We have demonstrated that Ang-(1–7) is able to induce the phosphorylation of GSK-3 β in the liver, adipose tissue and skeletal muscle as well as that of AS160 in skeletal muscle and adipose tissue of the rat *in vivo* [91,92]. The proline-directed serine/threonine kinase GSK-3 β , being one of the principal downstream targets of Akt, regulates a wide range of cellular processes, including glycogen metabolism, gene transcription, protein translation and cell apoptosis [126]. Under basal conditions, GSK-3 β is highly active and inhibits glycogen synthesis by phosphorylation of glycogen synthase [127]. Insulin-activated Akt phosphorylates GSK-3 β at the inhibitory

site Ser⁹, leading to stimulation of glycogen synthesis and protein synthesis [50]. AS160 is a Rab GTPase-activating protein and an important target of Akt [128]. It regulates insulin-stimulated GLUT4 trafficking [129] and glucose uptake [130]. The importance of AS160 in insulin-mediated glucose transport has been highlighted recently by the phenotype of AS160-KO (knockout) mice that display whole-body insulin resistance [131]. As a corollary of the discovery that Ang-(1–7) is able to induce the phosphorylation of Akt and AS160, a recent study has shown that Ang-(1–7) stimulates glucose transport in adipocytes [132]. Interestingly, this was associated with a decrease in NADPH oxidase expression and oxidative stress [132]. Some studies suggest that oxidative stress is able to impair PI3K and Akt insulin-signalling steps in cultured insulin-sensitive cell lines, such as 3T3-L1 adipocytes and L6 myocytes [133,134]. These results suggest a causal relationship between the Ang-(1–7)-mediated negative regulation of NADPH oxidase [with a resulting decrease in ROS (reactive oxygen species)] and an increase in glucose uptake. Thus the observed Ang-(1–7)-induced phosphorylation of GSK-3 β and AS160 is correlated well with the modulation of the action of insulin in terms of glucose metabolism.

FOXO1 is a transcription factor that regulates genes controlling many hepatic functions, including glucose production and lipid metabolism [135–137]. Under physiological conditions insulin inactivates FOXO1 by Akt-stimulated phosphorylation. This inactivation is required for adaptive nutrient homeostasis during periods of fasting and feeding [138]. During fasting, insulin levels fall and FOXO1 becomes dephosphorylated and translocates to the nucleus which stimulates catabolic processes to maintain glucose homeostasis. In the fed state, insulin levels increase leading to phosphorylation and inactivation of FOXO1 [138]. It has been shown recently that Ang-(1–7) stimulates the dephosphorylation of FOXO1 and consequently enhances its activity in human endothelial cells [93]. These findings suggest that Ang-(1–7) could be a fine-tuning regulator of insulin action through modulating the equilibrium between Akt and FOXO1 activation.

ANG-(1–7) COUNTERACTS THE NEGATIVE EFFECTS OF ANGIOTENSIN II ON THE ACTION OF INSULIN: POTENTIAL MOLECULAR MECHANISMS INVOLVED

There is ample evidence which indicates that Ang-(1–7) is able to counteract many of the haemodynamic and non-haemodynamic actions of AngII. The evidence accumulated over the last few years suggests that Ang-(1–7) acts as regulator of both glucose

control group, suggesting a state of insulin resistance. Administration of Ang-(1–7) normalized the response to glucose, thus enhancing insulin sensitivity. * $P < 0.05$ compared with the corresponding values in all other groups. (C) Table showing the metabolic parameters of fructose-fed rats chronically treated with saline, Ang-(1–7), A-779 or a mixture of Ang-(1–7) and A-779. Chronic administration of Ang-(1–7) reduced systolic blood pressure (SBP) and baseline plasma insulin and triacylglycerols in fructose-fed animals. In addition, concomitant administration of a Mas receptor blocker (A-779) blunted the beneficial effects exerted by Ang-(1–7). * $P < 0.05$ compared with the saline-treated mice. HOMA, homeostatic model assessment. The upper left-hand, upper right-hand and lower left-hand panels in (A), and (C) were reprinted from Regulatory Peptides, 177 (1–3), Muñoz M. C, Giani J. F, Burgh V, Mayer M. A, Carranza A., Taira C.A and Dominici F. P. The Mas receptor mediates modulation of insulin signaling by angiotensin-(1–7), 1–11, Copyright (2012), with permission from Elsevier. Copyright © 2012 Elsevier. The lower right-hand panel in (A) was reproduced from [89] with permission from The American Physiological Society. (B) was reproduced from [85] with permission from The American Physiological Society.

and lipid metabolism [36,37,39,139]. In this section we will discuss recent findings that show that the counteraction of the actions of AngII exerted by Ang-(1–7) also extends to the arena of metabolism. The first evidence originated from an *in vivo* analysis that revealed that in the rat heart Ang-(1–7) counteracts the negative effects of AngII [89]. Strikingly, the AngII-induced inhibition of insulin-stimulated Akt phosphorylation was no longer evident when Ang-(1–7) was co-injected with AngII [83]. Later, we found that this counteracting effect was detected in the main insulin-target tissues, namely the liver, skeletal muscle and adipose tissue [86] (Figure 3A). It was found that Ang-(1–7) not only counteracted the AngII-mediated inhibition of insulin-stimulated Akt phosphorylation, but also improved the phosphorylation of other insulin-related intracellular proteins, such as AS160 and GSK-3 β [86]. In a recent report, this counteraction of the inhibitory effects of AngII on insulin-induced Akt activation was confirmed using both human umbilical vein endothelial cells [140] and isolated rat soleus muscle [141]. Importantly, in skeletal muscle, Ang-(1–7) was shown to ameliorate the inhibitory effect of AngII on glucose transport activity [141]. Although the negative effects of AngII on insulin signalling are mediated by the AT₁R, the beneficial and counteracting effect of Ang-(1–7) on the actions of AngII at this level appear to be mediated by its specific receptor Mas [92,140,141] (Figure 3A).

AngII has been proposed as one of the most important inhibitors of the insulin signalling pathway. Evidence of this is the tight association that exists between the development of Type 2 diabetes and hypertension [142]. Indeed, the observation that antagonists of the AT₁R and ACE inhibitors not only reduce blood pressure, but also ameliorate insulin resistance is proof of the negative influence that AngII exerts over insulin signalling [19]. AngII inhibits insulin signalling at multiple levels [8–13,24,25]. For instance, AngII augments the phosphorylation of serine residues of the IR in aortic smooth muscle cells, leading to a significant reduction in insulin-mediated PI3K activation. [8,9]. However, serine phosphorylation of IRS-1 appears as one of the most important mechanisms of the AngII-mediated attenuation of insulin signalling. This negative effect of AngII is mediated by the AT₁R with important participation of ROS generation [143] (Figure 2). In fact, AngII has been shown to decrease insulin sensitivity in skeletal muscle and reduce glucose transport through ROS generation. A pivotal role has been assigned for NADPH oxidase in these events [144–146]. AngII impairs eNOS activation through ERK1/2-mediated phosphorylation of IRS-1 at Ser⁶¹² (human Ser⁶¹⁶) and JNK1/2-mediated Ser³⁰⁷ phosphorylation [24]. Alternatively, studies suggest that AngII induces the activation of p38 MAPK [147,148]. This enzyme has been suggested as a potential candidate for mediating indirect IRS-1 serine phosphorylation [149]. In VSMCs, AngII has been shown to decrease IRS-1 protein levels via Src, PDK1 and ROS-mediated phosphorylation of IRS-1 at Ser³⁰⁷ and the its subsequent proteasome-dependent degradation [150]. This evidence indicates that AngII-induced generation of ROS through an AT₁R/NADPH oxidase-dependent mechanism is linked to serine phosphorylation of the IRS proteins and acts a major contributing factor to AngII-induced insulin resistance. Finally, it has recently been shown that rapamycin, an inhibitor of mTOR, attenuates the

AngII-stimulated phosphorylation of p70S6K (p70 S6 kinase) and phosphorylation of IRS-1 (at Ser⁶³⁶ or Ser⁶³⁹) and blocks the ability of AngII to impair insulin-stimulated phosphorylation of eNOS and NO production [25]. In addition to mTOR and JNK, AngII-induced ERK1/2 activation has been shown to inhibit insulin-dependent glucose uptake through serine phosphorylation of IRS-1 in RASMCs (rat aortic smooth muscle cells) [151]. In the vasculature, AngII has been shown to inhibit Akt phosphorylation specifically through PKC- α activation [152].

One of the mechanisms by which Ang-(1–7) counteracts AngII-mediated insulin resistance appears to be the reduction in serine phosphorylation of IRS-1 (Figure 4). In the animal model of insulin resistance generated by a high-fructose diet in rats, we have demonstrated that Ang-(1–7) improves insulin signalling in the liver, skeletal muscle and adipose tissue [85]. This improvement was associated with a significant reduction in the phosphorylation of IRS-1 at Ser³⁰⁷ together with less activation of the kinases mTOR and JNK in skeletal muscle and adipose tissue [85]. In addition, Ang-(1–7) has been shown to counteract AngII signalling, leading to ERK1/2 and PKC activation both in proximal tubular cells and VSMCs [153–155], as well as in the rat heart [90] (Figure 2). Additional evidence for this mechanism was recently provided using human umbilical vein endothelial cells [140]. Accordingly, it was demonstrated that AngII induces the phosphorylation of IRS-1 at Ser⁶¹⁶ that was associated with an impairment of insulin signalling [140]. Importantly, this impairment was restored by Ang-(1–7) concomitant with a reduction in phosphorylation of IRS at Ser⁶¹⁶ [140]. Thus a reduction in serine phosphorylation of IRS-1 through a mechanism that involves inhibition of JNK, mTOR, and, potentially, ERK1/2 and PKC appears to play a key role in the counteraction of AngII-mediated inhibition of insulin signalling induced by Ang-(1–7). This improvement in insulin signalling may also involve suppression of oxidative stress as was shown previously [126] (Figure 4).

Although indirectly, the vasodilation induced by Ang-(1–7) could be considered as a possible contributing factor to its facilitating effect on the action of insulin (Figure 4). The delivery of insulin to the peripheral tissues is an important factor in the execution of its actions [156–158]. Thus Ang-(1–7) could potentiate the action of insulin both by enhancing vasodilation and counteracting the vasoconstrictive effects of AngII. This would result in enhanced insulin delivery to target tissues (Figure 4). In fact, a recent study has shown that blockade of the AT₁R with losartan increases muscle insulin delivery and counteracts the negative effects on insulin sensitivity associated with infusion of lipids [159].

THERAPEUTIC IMPLICATIONS

Previous reports have indicated that the modulation of ACE2/Ang-(1–7)/Mas receptor axis is a very attractive target in the therapy of the metabolic syndrome and diabetes-associated diseases affecting the heart and the kidney. These findings are summarized in Table 1. We found that chronic infusion of Ang-(1–7) ameliorates insulin resistance by reducing the levels of

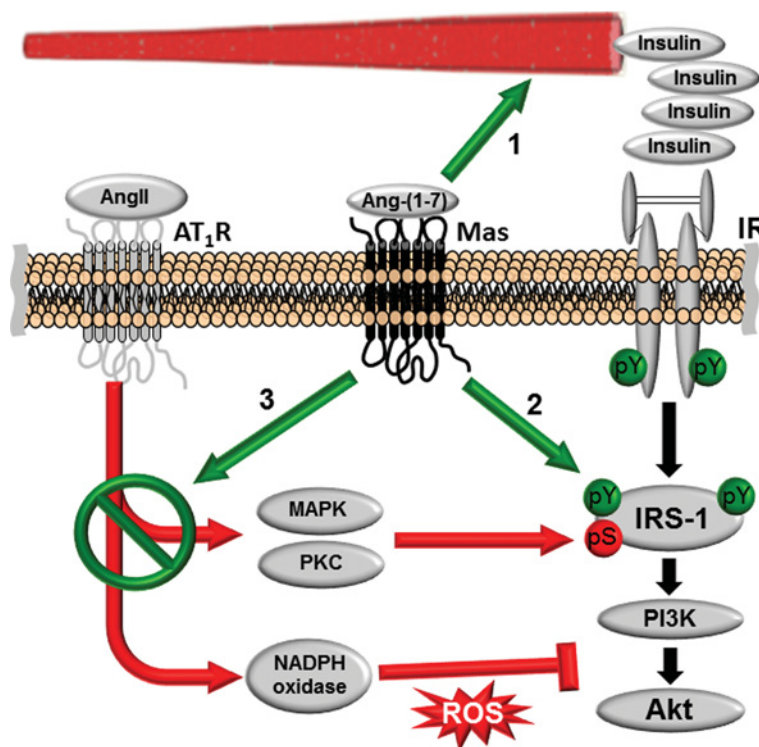


Figure 4 Potential mechanisms involved in the modulation of the action of insulin by Ang-(1-7) through its specific receptor Mas

1: Vasodilator effects increase the blood flow facilitating insulin delivery to peripheral tissues. 2: Direct stimulation of IRS-1 tyrosine phosphorylation. 3: Inhibition of the negative cross-talk between AngII and insulin signalling (serine/threonine kinases involved in serine phosphorylation of IRS-1 and production of ROS).

triacylglycerols and improving insulin sensitivity in fructose-fed rats [85] (Figures 3B and 3C). In a recent study these findings were confirmed and it was also demonstrated that Ang-(1-7) treatment prevents the metabolic-syndrome alteration, reduces ectopic lipid accumulation in the liver and diminishes the volume of epididymal adipocytes as well as the total body mass induced by fructose overload [160]. In agreement with these reports, transgenic rats overexpressing Ang-(1-7) display enhanced glucose tolerance and improved insulin sensitivity concomitant with enhanced insulin-stimulated glucose uptake in adipocytes and reduced triglyceridaemia, cholesterolaemia and abdominal fat mass [161]. Further efforts in characterizing the phenotype of these animals demonstrated the presence of reduced hepatic gluconeogenesis [162], as well as decreased susceptibility to the development of inflammation in adipose tissue [163]. This improvement in glucose and lipid metabolism induced by Ang-(1-7) correlates well with its capability for stimulating insulin signalling [88,89,91,92] and glucose transport [132]. These promising pre-clinical studies suggest that modulation of the ACE2/Ang-(1-7)/Mas receptor axis could improve haemodynamic and metabolic diseases in humans. Several meta-analyses have underscored the positive effects of ARBs (angiotensin receptor blockers) and ACE inhibitors on insulin sensitivity and the progression to Type 2 diabetes [164,165]. Since the ACE2/Ang-(1-7)/Mas receptor axis naturally counterbalances the effects of classical RAS components, it is reasonable to believe that part of the positive effects of ARBs and ACE inhibitors on metabolic

diseases could be mediated by overactivation of the Ang-(1-7) pathway. Indeed, a recent study has suggested that the beneficial effects of olmesartan, an ARB, on vascular remodelling are mediated via activation of the ACE2/Ang-(1-7)/Mas receptor axis [166].

A role for Ang-(1-7) in metabolism is also provided by manipulation of ACE2. Accordingly, ACE2-KO mice display normal insulin sensitivity when fed on a standard diet. However, AngII infusion or administration of an HFHS (high-fat high-sucrose) diet resulted in accentuated glucose intolerance and greater impairment of insulin sensitivity in these animals [167]. Importantly, this negative effect was blunted by administration of Ang-(1-7) [167]. In *db/db* mice, overexpression of ACE2 in the pancreas significantly improved fasting glycaemia, enhanced intraperitoneal glucose tolerance, increased islet insulin content and β -cell proliferation, and reduced β -cell apoptosis [168]. These findings identified ACE2 as a novel target for the prevention of β -cell dysfunction and apoptosis, both hallmarks of the onset of Type 2 diabetes. Moreover, a recent study has determined that maintenance of a normal endogenous ACE2 compensatory activity in the pancreas appears critical to avoid the β -cell dysfunction induced by an overactive RAS [169]. In AngII-infused mice exhibiting hyperglycaemia, hyperinsulinaemia and impaired glucose-stimulated insulin secretion from pancreatic islets, treatment with adenovirus expressing human ACE2 restored pancreatic ACE2 expression and prevented AngII-mediated elevated glycaemia, thus improving β -cell function [169].

Table 1 Interventions in the ACE2/Ang-(1–7)/Mas receptor axis leading to improvement of metabolic syndrome, diabetes-associated pathologies and obesityHDL, high-density lipoprotein; NF- κ B, nuclear factor κ B.

Pathology	Experimental approach	Effect
Metabolic syndrome	Chronic infusion of Ang-(1–7) into fructose-fed rats	Reduced serum triacylglycerols, improved glucose tolerance and insulin sensitivity [85], and reduced hepatosteatosis, epididymal adipocyte volume and total fat mass [160]
	Ang-(1–7) overexpression in transgenic rats	Enhanced glucose tolerance, insulin sensitivity and insulin-stimulated glucose uptake in adipocytes, reduced triglyceridaemia, cholesterolaemia and abdominal fat mass [161], down-regulation of hepatic gluconeogenesis [162], and resistance to high-fat diet with transgenic rats showing increased HDL-cholesterol levels, decreased abdominal fat mass and lower body mass compared with the wild-type animals [163]
	Acute Ang-(1–7) stimulation in rats	Activation of the insulin signalling-related proteins IRS-1, Akt, GSK3 and AS160 via the receptor Mas [88,89,91–93]
	Ang-(1–7) treatment of adipocytes	Improved glucose uptake both in basal and insulin-stimulated states via the Mas receptor and reduced ROS production [132]
	Deletion of ACE2 in mice	Normal insulin sensitivity when fed on a standard diet, but, increased susceptibility to AngII infusion or when fed on a high-fat and high-sucrose diet in terms of the development of glucose intolerance and impairment of insulin sensitivity; this negative effect was eradicated by Ang-(1–7) [167]
	Overexpression of ACE2 in the pancreas of <i>db/db</i> mice	Improved fasting glycaemia, enhanced intraperitoneal glucose tolerance, increased islet insulin content and β -cell proliferation, and reduced β -cell apoptosis; effect eliminated by A-779 [168]
	Overexpression of ACE2 in AngII-infused mice	AngII-infused mice exhibited hyperglycaemia, hyperinsulinaemia and impaired glucose-stimulated insulin secretion from pancreatic islets, decreased ACE2 expression and activity, increased AT ₁ R expression, and increased oxidative stress in the pancreas; adenovirus treatment encoding human ACE2 restored pancreatic ACE2 expression, improved β -cell function and restored glucose homeostasis [169]
	Mas-KO mice	Dyslipidaemia, increased levels of insulin, leptin and abdominal fat mass with normal body mass, glucose intolerance, reduced insulin sensitivity and insulin-stimulated glucose uptake by adipocytes and decreased GLUT4 in adipose tissue [171]
	Adipocytes from Mas receptor-knockout male mice	Altered response of adipocytes to insulin action; effect related to decreased expression of PPAR γ [172]
Diabetes-induced cardiovascular dysfunction	Chronic administration of Ang-(1–7) or AVE-0991 on diabetic rats	Prevention of diabetes-induced abnormal vascular responsiveness to noradrenaline, endothelin-1, AngII, carbachol and histamine in the mesenteric bed, isolated carotid and renal arteries [173]; increased LV-developed pressure and serum nitrite/nitrate and decreased LV mass/body mass and LV collagen content [174]; AVE-0991 rescued cardiac function under diabetic conditions as indicated by a normalization of blood pressure and contractility parameters [175]; and Ang-(1–7) inhibited EGF receptor transactivation via a Mas receptor/Src-dependent pathway, indicating that activation of the EGF receptor is a key player in mediating diabetes-induced vascular dysfunction [177]
	Chronic infusion of XNT (ACE2 activator) on STZ-induced diabetic rats	Improved endothelium-dependent vasorelaxation of aortic rings; effect blocked by A-779 [176]
	Deletion of ACE2 in diabetic Akita mice	Increased plasma and tissue AngII, impaired systolic and diastolic function, increased activation of NADPH oxidase, and greater oxidative stress [178]
	Chronic infusion of Ang-(1–7) into fructose-fed rats	Reduced blood pressure, heart-to-body mass ratio, myocyte diameter and LV fibrosis [179]
Diabetic nephropathy	Chronic infusion of Ang-(1–7) on STZ-treated spontaneously hypertensive rats	Reduction of renal NADPH oxidase activity and proteinuria, and attenuation of diabetes-induced increase in the renal vascular responsiveness to endothelin-1 [180]
	Chronic infusion of Ang-(1–7) into STZ-induced diabetic rats	Attenuated proteinuria and renal collagen content and improved endothelial functions without preventing tubular damage; effects blocked by A-779 [181]
	Chronic infusion of Ang-(1–7) into KK-A y /Ta diabetic mice	Improved mesangial expansion, attenuated AngII-mediated NADPH oxidase activation and ROS production in glomeruli and mesangial cells, and attenuated AngII-induced NF- κ B and MAPK signalling in mesangial cells [182]
	Chronic infusion of Ang-(1–7) into Zucker diabetic fatty rats	Reduced proteinuria and systolic blood pressure, restored creatinine clearance, and decreased renal fibrosis, oxidative stress and inflammatory cytokines [184]

Table 1 Continued

Pathology	Experimental approach	Effect
Diabetic retinopathy	Deletion of ACE2 in mice	Exaggerated diabetic nephropathy induced by STZ administration [185]
	Overexpression of ACE2 in podocytes of diabetic mice	Prevented albuminuria, attenuated the increase in the mesangial area and the decrease in glomerular area, and restored nephrin expression [186]
	Inhibition of ACE2 in diabetic rats	Exacerbation of diabetes-induced increase in cardiac and renal NADPH activity [187]
	Ang-(1–7) treatment of endothelial progenitor cells (CD34 ⁺) from patients with diabetes	Restored migration and NO bioavailability/cGMP production via Mas receptor and decreased NADPH oxidase activity, and enhanced survival and proliferation of CD34 ⁺ cells [188]
Diabetic wounds	NorLeu(3)-Ang-(1–7) [analogue of Ang-(1–7)] treatment of diabetic wounds	Induced progenitor cell proliferation and accelerated vascularization, collagen deposition and re-epithelialization [189]
Obesity	Administration of an oral formulation of Ang-(1–7)	Prevention of obesity, hepatic inflammation and hepatic steatosis induced by a high-fat diet, and improvement in lipid metabolism [191,192]

It is worth mentioning that ACE inhibitors fail to completely block AngII formation since many other alternative proteases can still produce AngII and generate hyperglycaemia in the presence of ACE inhibitors [170]. ACE2, on the other hand, acts directly on AngII, irrespective of the pathways involved in the production of this octapeptide. Thus, by ensuring depletion of AngII in the body, ACE2 therapy also increases the possibility of Ang-(1–7) emerging as a more efficient treatment than ACE inhibition in combating AngII-mediated hyperglycaemia [47,168,169]. The safety and tolerability, as well as the pharmacokinetics and pharmacodynamics of intravenous administration of recombinant soluble human ACE2 (known as APN01), is under evaluation currently [Safety and Tolerability Study of APN01 (Recombinant Human Angiotensin Converting Enzyme 2); ClinicalTrials.gov Identifier: NCT00886353].

Confirmatory evidence of a role for the ACE2/Ang-(1–7)/Mas receptor axis in glucose and lipid homeostasis was provided by the characterization of the phenotype of Mas-KO mice [171]. Mas-KO mice displayed dyslipidaemia and increased levels of insulin, leptin and abdominal fat mass. In addition, these mice show glucose intolerance, reduced insulin sensitivity, insulin-stimulated glucose uptake in adipocytes and decreased GLUT4 expression in adipose tissue [171]. An additional study determined that adipocytes from Mas-KO mice exhibit a blunted response to insulin in terms of a decrease in glycerol release, suggesting that the lack of Ang-(1–7) action through the Mas receptor alters the response of adipocytes to insulin action. These effects could be related to the decreased expression of PPAR γ (peroxisome-proliferator-activated receptor γ) in the adipocytes of Mas-KO mice [172].

Owing to the demonstrated role of Ang-(1–7) as a facilitator of the action of insulin and as a negative regulator of the action of AngII, several studies have analysed its beneficial effects on pathologies associated with Type 2 diabetes. An important area of research is the treatment of diabetes-induced cardiovascular dysfunction. In this regard, it has been demonstrated that chronic administration of Ang-(1–7), or its synthetic analogue AVE-0991, prevented the diabetes-induced abnormal vascular responsiveness to noradrenaline, endothelin-1, AngII, carbachol and histamine in the mesenteric bed, isolated carotid and renal arteries of rats

made diabetic by treatment with STZ (streptozotocin) [173]. Further studies in STZ-treated rats demonstrated that treatment with Ang-(1–7) increases LV (left ventricular)-developed pressure and serum nitrite/nitrate, and decreases the LV mass/body mass ratio and LV collagen content in diabetic animals [174]. In agreement with these studies, treatment with AVE-0991 has been shown to rescue cardiac function under diabetic conditions as indicated by a normalization of blood pressure and contractility parameters [175]. More recently, it was shown that chronic infusion of XNT {1-[(2-dimethylamino) ethylamino]-4-(hydroxymethyl)-7-[(4-methylphenyl) sulfonyl oxy]-9H-xanthene-9-one; an ACE2 activator} into STZ-induced diabetic rats improves the endothelium-dependent vasorelaxation of aortic rings. This effect was blocked by the Mas receptor antagonist A-779 [176]. In general these studies imply a cardioprotective role for Ang-(1–7) under hyperglycaemic conditions and point to new therapeutic strategies using Ang-(1–7) agonists to treat the cardiovascular complications associated with diabetes mellitus. A potential mechanism involved in these beneficial effects of Ang-(1–7) could be related to its ability to inhibit EGF (epidermal growth factor) receptor transactivation via a Mas receptor-dependent pathway [177]. In addition, an indirect role for this cardioprotective role of Ang-(1–7) has been demonstrated by deletion of the *Ace2* gene in diabetic Akita mice. These animals displayed increased plasma and tissue AngII, impaired systolic and diastolic function, and increased NADPH oxidase activity and greater oxidative stress in the heart [178]. A protective effect for Ang-(1–7) on cardiac function has also been demonstrated in fructose-fed rats, where chronic infusion of Ang-(1–7) reduced blood pressure, heart-to-body mass ratio, myocyte diameter and LV fibrosis. These beneficial effects were associated with a reduced activity of growth-promoting signalling molecules pathways (i.e. ERK1/2, JNK1/2 and p38 MAPK) in the heart. [179].

Diabetic nephropathy is another major complication of Type 2 diabetes and is a main cause of end-stage renal disease. In line with its beneficial effect on the action of insulin, Ang-(1–7) has been shown to have a renoprotective effect in both diabetic and insulin-resistant rats. In diabetic spontaneously hypertensive rats, chronic infusion of Ang-(1–7) decreased the elevated levels of renal NADPH oxidase activity, reduced proteinuria

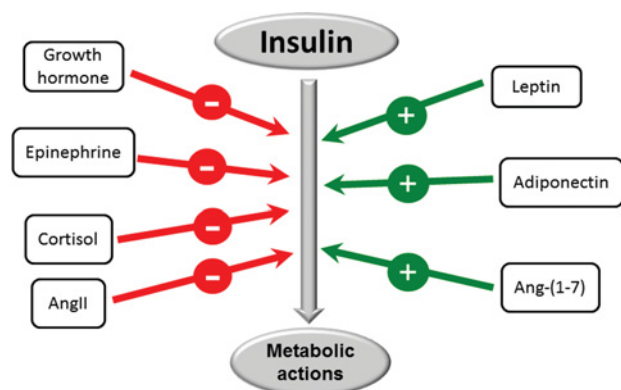


Figure 5 Modulation of insulin-mediated metabolic actions by different hormones

and attenuated the diabetes-induced increase in renal vascular responsiveness to endothelin-1 [180]. In STZ-treated diabetic rats, chronic infusion of Ang-(1-7) attenuated proteinuria and renal collagen content. This correlated with an improvement in endothelial function. Such beneficial effects were blocked by A-779 [181]. A study using KK-A^y/Ta mice demonstrated that chronic infusion of Ang-(1-7) improves mesangial expansion and attenuates AngII-mediated NADPH oxidase activation and ROS production in glomeruli and mesangial cells [182]. Ang-(1-7)-induced attenuation of renal NADPH oxidase has also been shown to correlate with the preservation of PPAR γ and catalase activity [183]. We have demonstrated that treatment of Zucker diabetic rats with Ang-(1-7) reduces proteinuria and systolic blood pressure, restores creatinine clearance, and decreases renal fibrosis, oxidative stress and inflammatory cytokines [184]. Highlighting the role of Ang-(1-7), ACE2-KO mice displayed accelerated time-dependent glomerular and tubulointerstitial damage when treated with STZ [185]. In good agreement with this finding, overexpression of ACE2 in the podocytes of diabetic mice was recently shown to prevent microalbuminuria, attenuate the increase in mesangial area, decrease the glomerular area and restore nephrin expression [186]. Confirmation of the beneficial role of endogenous Ang-(1-7) in the cardiovascular system was provided by a study where chronic treatment with the ACE2 inhibitor DX600 exacerbated the diabetes-induced increase in cardiac and renal NADPH activity [187]. Finally, modulation of the ACE2/Ang-(1-7)/Mas receptor axis appears to be an attractive therapeutic target in the treatment of diabetic retinopathy [188] and also has a potential application as an accelerator of vascularization and re-epithelization [189].

In view of the great therapeutic effect of the modulation of the ACE2/Ang-(1-7)/Mas receptor axis, a formulation of Ang-(1-7) bound in a cavity in the oligosaccharide HP β CD23 (hydroxypropyl β -cyclodextrin 23) that protects the peptide, when orally administered, during passage through the gastrointestinal tract has been developed [190]. Recently, this oral formulation of Ang-(1-7) has been shown to counteract the deleterious effects of a high-fat diet [191,192], supporting its potential application in the treatment of the metabolic syndrome and diabetic complications.

CONCLUSIONS AND PERSPECTIVES

The overall knowledge of the physiological and pathophysiological role of Ang-(1-7) has advanced greatly in the last decade. In particular, it has become clear that Ang-(1-7) counteracts both the cardiovascular and non-cardiovascular actions of AngII. Recent findings have shown that this counteraction applies to the modulation of the action of insulin in glucose and lipid metabolism. Potential mechanisms by which this beneficial effect is exerted include direct activation of insulin signalling, inhibition of the negative actions of AngII and increased delivery of insulin to the target tissues. Thus Ang-(1-7) should be considered as a member of the group of hormones that can modulate the action of insulin, in this case positively (Figure 5). The implications of these findings are beginning to be revealed, and make modulation of the ACE2/Ang-(1-7)/Mas receptor axis a very attractive therapeutic option in the treatment of the metabolic syndrome and pathologies associated with Type 2 diabetes.

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