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Pathophysiological role of the renin–angiotensin system on erectile dysfunction

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ABSTRACT

Background The renin–angiotensin system (RAS) has been shown to play an active role within the erectile tissues. The aim of this narrative review is to summarize the literature addressing the pathophysiological role of RAS on erectile function. Additionally, we update evidence on recent findings on the role of the Ang-(1-7) and Mas receptor on the erectile function and its therapeutic potential for treating erectile dysfunction (ED).

Materials and methods This narrative review is based on the material searched and obtained via MEDLINE and PubMed up to November 2012. The search terms we used are 'angiotensin, erectile dysfunction, renin, Mas receptor' in combination with 'pathophysiology, fibrosis, pathways'.

Results The levels of angiotensin (Ang) II, the main component of this system, are increased in the corpus cavernosum as compared to those found in the systemic circulation. Moreover, emerging evidence indicates that an increased activity of Ang II via AT1 receptor might contribute to the development of ED, whereas the pharmacological blockage of Ang II/AT1 actions has beneficial effects on the erection. On the other hand, the heptapeptide Ang-(1-7), known as a major endogenous counter-regulator of Ang II actions, favours penile erection via the activation of Mas receptor.

Conclusions Ang-(1-7) and Mas receptor pathway might be considered as a promising therapeutic target for the treatment of ED.

Keywords Angiotensin II, angiotensin-(1-7), erectile dysfunction, Mas receptor, renin–angiotensin system.

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Introduction

Erectile dysfunction (ED) is a widespread multicausal disorder that affects millions of human beings worldwide [1,2]. In the Massachusetts Male Ageing Study, 52% of men reported some degree of ED, with the probability of experiencing complete ED tripling from 5% to 15% between the ages of 40 and 70 years [3]. Over 7 million patients in EU and US suffer from severe ED that is nonresponsive to oral pharmaceuticals [4]. Additionally, the prevalence of ED is expected to rise considerably over the next 25 years, impacting more than 300 million people by 2025 [2].

Penile erection is a coordinated neurovascular response [1]. In a flaccid state, penile smooth muscles are tonically contracted, allowing only a small amount of blood flow for nutritional purposes. Penile erection occurs when sexual stimulation triggers the release of neurotransmitters, mainly nitric oxide

(NO) from the cavernous nerve terminals. The neurotransmitters cause relaxation of smooth muscle cells in cavernosal arterioles and sinuses, resulting in an increased blood flow into the penis. Subsequently, endothelial-derived NO release is mediated by the activation of shear stress-dependent stimulation of the cavernous endothelial lining [1]. These events cause the cavernous sinuses to fill with blood and expand against the tunica albuginea, partially occluding the venous outflow, thus resulting in erection formation [1].

The major mechanisms responsible for ED are the failure in the neuronal response and/or the increase in tone and/or contractility of the smooth muscle within the corpus cavernosum and penile arteries [1,5]. Several pharmacological treatments against ED are currently available. Such drugs, however, are not entirely effective and may present severe side effects [6].

Depending on the severity of ED and/or any underlying disease, sildenafil taken orally is effective for up to 70% of patients in producing an adequate erection for sexual intercourse [4]. Sildenafil is a phosphodiesterase-5 (PDE-5) inhibitor, which suppresses the enzyme responsible for breaking down the intracellular second messenger cGMP generated by NO stimulus. cGMP is involved in the regulation of several protein-dependent kinases, which mediate smooth muscle cells relaxation and thus facilitate erection [4]. The PDE-5 inhibitor effect is dependent on NO release by cavernosal nerves; thus, it is not effective unless the neuronal stimulus has been initiated and becomes functional [4]. Patients, who cannot be treated with PDE-5 inhibitors or find them ineffective, may achieve the erection using intrapenial injection of vasodilators [7,8]. Alprostadil (Prostaglandin E1, PGE1) is the most common vasodilator used for ED [8,9]. It can be administered by direct injection into the corpus cavernosum with a needle or inserted into the urethra in pellet form through a delivery system [10]. Alprostadil has been shown to be effective in over 80% of patients with ED [8,10]; however, the use of intrapenial injections is quite problematic for several side effects, such as, penile pain, bleeding, priapism, haematoma and penile fibrosis, which can lead to permanent ED [7,8]. Thus, there is clearly a great need for more effective modalities for achieving an adequate erection in patients suffering from ED.

The renin-angiotensin system (RAS) is a well-known modulator of cardiovascular homeostasis [11,12]. Moreover, there is strong evidence that RAS plays a critical role in erectile function [13–18]. Besides endocrine actions, the RAS is expressed within the cavernosal tissue and may act in a paracrine manner modulating corpus cavernosum smooth muscle cell contraction and tone [13]. In fact, the physiological amount of angiotensin II (Ang II) produced in erectile tissues is significantly higher than those found in the systemic circulation [14], indicating an intense activity within the erectile tissue.

In the 'traditional' paradigm, the RAS is orchestrated by a series of enzymatic reactions culminating in a linear generation and action of Ang II [11]. In the pathological condition, this peptide has been shown to bind AT1 receptor and produce potentially deleterious effects, such as vasoconstriction, proliferation, fibrosis and oxidative stress [19–22]. Within the corpus cavernosum, Ang II modulates smooth muscle cell contraction and tone [13–15,23,24]. Moreover, the hyperactivity of this peptide is closely associated with the pathogenesis of ED [14].

In the last decade, the concept of RAS as 'a linear cascade' has been expanded, mostly due to the discovery of key additional components, such as angiotensin-(1-7) [Ang-(1-7)], angiotensin-converting enzyme 2 (ACE2) and the Mas receptor [20–22]. Ang-(1-7) is a bioactive member of the RAS, which activates the Mas receptor [25] and promotes many beneficial cardiovascular outcomes, such as vasodilation, NO release and

antiproliferative and antifibrotic effects [20,22,25]. Within the erectile tissues, in an opposite way to Ang II, Ang-(1-7) has been shown to favour the erection (Fig. 1) [17,18].

In this narrative review, we will summarize the activities of RAS in male ED pathophysiology. In addition, we will highlight the recent findings addressing the role of the Ang-(1-7) and Mas receptor on the ED and its therapeutic potential. This narrative review is based on the material searched for and obtained via MEDLINE and PubMed up to November 2012. The search terms we used were 'angiotensin, erectile dysfunction, renin, Mas receptor' in combination with 'pathophysiology, fibrosis, pathways'.

Update on the current view of the renin-angiotensin system

The RAS is a peptidergic hormonal system, which plays a key role in the pathophysiology of cardiovascular [22,26] and erectile disease [13–18]. In a classical concept, the RAS biosynthesis consists of a sequential linear enzymatic reactions, initiated by the conversion of the precursor angiotensinogen to the inactive decapeptide Ang I, mediated by renin [22]. Sequentially, ACE cleaves Ang I and forms the octapeptide Ang II, the main effector of RAS [22,26,27]. Ang II exerts its biological

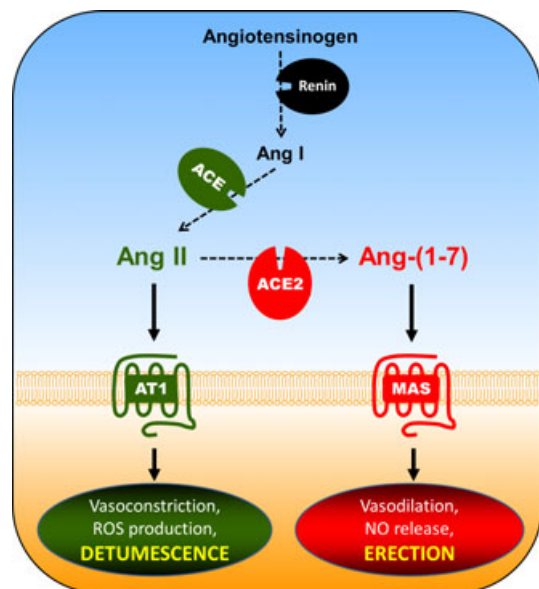


Figure 1 Schematic representation of the renin-angiotensin system (RAS) cascade and its role on erectile function. Focus on the two major axes of the RAS and its major effects on erectile tissues. Ang I, angiotensin I; Ang II, angiotensin II; Ang-(1-7), angiotensin-(1-7); ACE, angiotensin-converting enzyme; ACE2, angiotensin-converting enzyme 2; AT1, angiotensin II type 1 receptor; Mas, Mas receptor.

effects by the activation of two distinct G protein-coupled receptors (GPCR), AT1 and AT2 [22,26,28]. In both physiological and pathological conditions, AT1 is involved in the Ang II-mediated vasoconstriction, proliferation, fibrosis and inflammation [11,20]. Oppositely, AT2 is associated with vasodilation, NO production and antiproliferative and anti-inflammatory effects, thus antagonizing AT1 effects [12,19]. Therefore, AT2 activation may produce beneficial actions in several diseases; however, its role on penile erection remains uncertain. In fact, Park and co-workers [24] showed that the specific binding of Ang II in the corpus cavernosum of rabbit was displaced by a selective AT1 antagonist, but not by AT2 antagonist, suggesting a marginal role of the AT2 receptors in the erectile tissues. However, considering the paucity of studies investigating the interactions between AT2 and AT1 receptors especially in ED, we can only speculate on this aspect. Interestingly, the potential AT2-mediated counter-regulation of AT1 bioactivities (antiproliferative vs. growth action) was firstly suggested by Nakajima and co-workers in 1995 in cultured smooth muscle cells [29]. Then, Suzuki and colleagues more recently confirmed these findings on direct counterbalance between AT1 and AT2 on vascular cells performing an *in vivo* study using a mouse model of vascular injury and neointimal formation [30]. The treatment potential targeting this relevant receptor interference remains to be elucidated and importantly contributed to the recent increase in RAS pathophysiological complexity.

In fact, in the last decade, the classical RAS paradigm as a linear cascade leading to Ang II formation and actions has been expanded and became very complex [20]. A counter-regulatory pathway in which Ang-(1-7) acts as the main effector [20,22] has been proposed. Ang-(1-7) is a heptapeptide, the actions of which are frequently opposite to those attributed to Ang II [31]. Acting through the Mas receptor [25], Ang-(1-7) produces vasodilation [32], NO release [33] and antiproliferative [34], antifibrotic [35,36] and anti-inflammatory [37] effects, and is considered as the main endogenous counter-regulator of Ang II effects [20,22,31]. Ang-(1-7) is mainly produced by ACE2, which cleaves the C-terminal phenylalanine of Ang II, leading to Ang-(1-7) formation [38,39]. In addition, neutral endopeptidase (such as prolylcarboxypeptidase [PCP] and prolylendopeptidase [PEP]) may also be involved in the generation of Ang-(1-7) [39–41].

Currently, it is well accepted that the RAS is modulated by two opposite branches, one deleterious triggered by Ang II/AT1 receptor and the other protective triggered by Ang-(1-7)/Mas receptor [42]. Evidence suggests that these two branches might contribute to the cardiovascular homeostasis, and a chronic and sustained imbalance between them may contribute to the development of pathologies [43]. For instance, in erectile tissues, it was found that the elevated production of Ang II was associated with ED in humans [14].

Role of Ang II/AT1 receptor axis in penile erection physiology and ED pathophysiology

The RAS has been documented to be highly involved in disturbances of the cardiovascular system, being an important target to manage cardiovascular diseases (CVDs) [26,44,45]. Likewise, RAS is also considerably involved in ED pathophysiology [13,23,24].

Many organs and tissues express the components of RAS, and this system may act in a paracrine manner [43]. The existence of local RAS in the penis has been confirmed by numerous studies in animals and humans [13–15]. Indeed, AT1 receptor, ACE, Ang I, Ang II and Ang III were detected within the human corpus cavernosum [15]. Moreover, additional studies have shown that human corpus cavernosum produces and secretes physiological amounts of Ang II greater than those found in the systemic plasma [14], indicating an intense modulation of erectile tissue by RAS. Supporting these observations, Iwamoto and co-workers showed that ACE activity in canine corpus cavernosum was 30-fold higher than in canine common carotid artery [46].

Physiologically, Ang II mediates the tonic contraction of the smooth muscle in the corpus cavernosum [13,24]. The intracavernosal injection of the AT1 antagonist losartan increased the cavernosal pressure, confirming the fundamental role of Ang II in the maintenance of the penile flaccid state [23]. Furthermore, Ang II levels in the human corpus cavernosum were shown to be increased after erection had ceased, indicating an active role of this molecule in the detumescence process [14]. Most of the actions of Ang II are mediated by AT1 receptor activation. This receptor is well expressed on the cavernosal smooth muscle and endothelial cells of corpus cavernosum [23,47]. Stimulation of AT1 triggers multiple signal transduction pathways, leading to a variety of functions [28]. In smooth muscle cells, AT1 stimulation may cause vasoconstriction through a calcium-dependent and calcium-independent pathway [47]. AT1 activation increases the influx of extracellular Ca^{2+} and the mobilization of intracellular Ca^{2+} , culminating in the activation of myosin light chain kinase [47]. AT1 stimulation also activates RhoA/Rho-kinase pathway, leading to inhibition of myosin light chain phosphatase (MLCP) [48,49]. These independent processes suggest Ang II to be one of the most powerful endogenous physiological vasoconstrictors.

Angiotensin II type 1 receptor (AT1) stimulation might increase reactive oxygen species (ROS) production via activation of nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase [28]. Reactive oxygen species (ROS) serve as second messengers involved in physiological redox signalling pathways to regulate cellular functions; however, its overproduction might be closely associated with the development of vasculogenic ED [50]. In fact, it was documented that the

erection impairment caused by Ang II infusion was associated with an increase in NADPH oxidase expression and ROS production into the corpus cavernosum [51]. In addition, AT1 stimulation may also regulate various kinase pathways to induce cell proliferation, hypertrophy and fibrosis [52].

The augmented action of Ang II/AT1 receptor has been associated with many cardiovascular disorders as well as ED. It was found that Ang II plasma levels were elevated in the cavernous blood of patients with an organogenic aetiology of ED [14]. In this line, ACE mRNA expression was shown as up-regulated in a rat model of arteriogenic ED [53]. Others have found that Ang II levels in penile tissue from diabetic rats with ED were significantly higher than that in the control rats [54]. Reinforcing the correlation of Ang II/AT1 hyperactivity and ED, *in vivo* studies demonstrated that intrapenial injection of Ang II abolished spontaneous erections observed in anaesthetized dogs [15]. Interestingly, the ED animal model (mice and rat) based on subcutaneous infusion of Ang II has become common in the literature [51,55], highlighting the central role of Ang II/AT1 in the pathophysiology of ED.

Increasing evidence from these data indicated a positive correlation between Ang II and ED. Therefore, pharmacological treatments targeting Ang II inhibition might represent a promising strategy against vasculogenic ED. Angiotensin II type 1 receptor blockers (ARB) and angiotensin-converting enzyme inhibitors (ACEi) have been already shown to be beneficial on the sexual function [56–62]. In an animal model of hypertension-induced ED, captopril reversed the impairment of erectile function [57]. Accordingly, telmisartan and ramipril (ARBs and ACEi respectively), significantly improved the cavernosal endothelial function of hypercholesterolaemic mice via the reduction in oxidative stress [60]. Moreover, in a rat model of diabetes-induced ED, losartan treatment restored the impaired erectile function [61]. The positive effects of ARBs and ACEi on erectile function have also been documented in humans. Although many antihypertensive drugs have the inconvenient adverse effect of causing or worsening ED [63], it has been suggested that compounds blocking Ang II actions may less affect the sexual function or even improve it [58,59,64,65]. Fogari and colleagues compared the effect of valsartan or carvedilol on sexual activity in never-treated hypertensive men [58]. The authors observed that carvedilol induces a chronic worsening of sexual activity, while valsartan did not affect or may even improve the sexual activity [58]. Llisterri and co-workers reported that losartan improved both satisfaction and frequency of sexual activity of hypertensive patients with ED [59]. A similar beneficial effect of valsartan on sexual activity in hypertensive patients was also observed by other research groups [64]. Moreover, in diabetic patients, losartan significantly improved erectile function assessed by three quantification methods (such as International Index of

Erectile Function, the percentage of successful penetrations and intercourse completions, and the global assessment question) [56]. Interestingly, the association of losartan and tadalafil was more effective improving the sexual activity than the use of losartan or tadalafil alone [56]. The beneficial effect of the blockage of Ang II activities has also been reported in patients with ED caused by nerve-sparing radical retropubic prostatectomy. In these patients, the treatment with irbesartan significantly increased sexual activity [62]. In addition, the use of irbesartan appears to control the loss of stretched penile length, which occurs postoperatively [62]. In contrast with these results, a recent study pointed out a relatively high reporting of ED in association with ARB treatment, suggesting these drugs to have a negative effect on ED [66]. Further and larger clinical trials are needed to clarify this point.

Ang-(1-7)/Mas receptor axis in penile erection physiology and ED pathophysiology

Ang-(1-7) is accepted as the main endogenous counter-regulatory factor of Ang II [20,31]. Contrarily to Ang II, Ang-(1-7) produces vasodilation [32], decreases blood pressure [67], reduces oxidative stress [68,69] and has antithrombotic [70–72], antiproliferative [34] and antifibrotic actions [35,36]. In keeping with that, Ang-(1-7) appears to mediate penile erection. Costa Goncalves and co-workers described for the first time the potential pro-erectile effect of Ang-(1-7) both *in vivo* and *in vitro* [16]. Using a deoxycorticosterone acetate (DOCA)-salt model of hypertensive male Wistar rats (uninephrectomized, subcutaneously implanted with a silicone device containing DOCA and offered of 1% NaCl and 0.2% KCl to drink), the authors showed that acute injection of Ang-(1-7) into the corpus cavernosum potentiated the erectile response induced by electrical stimulation of the major pelvic ganglion. Interestingly, intracavernosal injection of A-779 (a Mas receptor antagonist) reduced Ang-(1-7)-mediated effects on erectile response in these animals, indicating an endogenous role of Ang-(1-7) in penile physiological erection. Accordingly, when authors evaluated the erectile response in *Mas* receptor knockout mice, they found it substantially reduced. In this line, it was observed that Ang-(1-7) produces concentration-dependent relaxation in rabbit corpus cavernosum strips [17]. Additionally, Ang II-induced contraction of the corpus cavernosum was increased in the presence of A-779, further supporting the fundamental role of Ang-(1-7)/Mas pathway in the regulation of penile erection [17].

Therefore, recent evidence suggests that the activation of Ang-(1-7)/Mas axis may ameliorate or even reverse vasculogenic ED. Likewise, very recently, we have shown that treatment with Ang-(1-7) prevented ED in a mouse model of hypercholesterolaemia-induced ED. Using oral formulation of

Ang-(1-7), we observed that chronic treatment with Ang-(1-7) reduced corpus cavernosum fibrosis, which was associated with an attenuation of oxidative stress [73]. Importantly, Ang-(1-7) improved the cavernosal endothelial function, possibly through augmented NO bioavailability [73].

The mechanisms underlying Ang-(1-7)-mediated protection are still poorly understood. To further increase complexity, this shorter angiotensin peptide was also indicated to potentially act as an endogenous ligand not only of Mas, but potentially of both AT1 and AT2 receptors [74–77]. In addition, Ang-(1-7) was shown to potentially influence both AT1 and AT2 receptors' activities on vascular and immune cells *in vitro* [78,79] and *ex vivo* in Langendorff-perfused mouse hearts [80]. *In vivo*, Ang-(1-7) was shown to abrogate Ang I- and Ang II-induced vascular modifications in Wistar–Kyoto rats through a direct blockade of AT1 receptor bioactivities [81]. Clark and colleagues suggested that Ang-(1-7)-mediated effects might be also due to the down regulation of AT1 receptor [82,83]. However, additional pathophysiological mediators might be potentially involved [84]. For instance, the increase of NO bioavailability might also play a relevant role in the different vascular responses and particularly in ED [18]. NO is the main effector in the erectile response, and impaired NO bioactivity is a major pathogenic mechanism of ED [85]. Most of the effects promoted by Ang-(1-7) are frequently related to NO release [20,21]. Acting through the Mas receptor, Ang-(1-7) stimulates eNOS phosphorylation/activation via Akt-dependent pathways in human endothelial cells, resulting in NO production [33]. In fact, both the relaxation of cavernosal strips produced by Ang-(1-7) and the *in vivo* potentiation of erectile response were completely blocked by N-nitro-L-arginine methyl ester (L-NAME) [17]. Furthermore, incubation of rat and mouse corpus cavernosum strips with Ang-(1-7) resulted in increased NO release, while electrical stimulated release of NO in the rat corpus cavernosum was potentiated by Ang-(1-7) [16]. Supporting these findings, the facilitatory effect of AVE-0991, a nonpeptide synthetic Mas agonist, was blocked by L-NAME [18]. Additionally, chronic treatment with Ang-(1-7) increased both eNOS and nNOS protein expression in our mouse model of hypercholesterolaemia-induced ED [73]. Taken together, these evidences support the augmented NO bioavailability as a crucial mechanism underlying Ang-(1-7)/Mas axis-mediated pro-erectile function; however, if Ang-(1-7)/Mas activation favour cavernous nerve terminals-derived NO, endothelial-derived NO or both remains unclear.

Another important documented property of Ang-(1-7) in the erectile tissues is represented by a potent antifibrotic activity. Indeed, Ang-(1-7) can prevent collagen deposition in various tissues, such as heart [86], kidney [87,88], lung [89], liver [90], as well the penis [73]. It was found that *Mas* receptor gene-deleted mice have a marked increase in the fibrous tissue within the

corpus cavernosum [16]. Recently, we observed that chronic treatment with Ang-(1-7) produced a significant reduction in the penile fibrosis of hypercholesterolaemic mice [73]. The antifibrotic effect of Ang-(1-7) has been associated with attenuation of cytokine signalling cascades [89], inhibition of MAPKs signalling cascades [91,92], modulation of matrix metalloproteinase activity [93] and a reduction in oxidative stress [36]. However, the mechanism by which Ang-(1-7) reduces fibrosis in the penis still remains unknown.

Perspectives and future directions

Overall, the discussion above supports the concept that the RAS system is critical in the pathophysiology of erectile function. Moreover, the blockade of Ang II/AT1 axis actions as well as the activation of Ang-(1-7)/Mas axis appears to be a potential pro-erectile target for treating ED. However, several critical issues regarding the role of RAS on erectile function still need to be clarified. Many key topics of investigation might be considered:

- 1 Many studies suggest that ARBs and ACEi might improve the sexual activity of patients with ED [56,58,59,62,64,65]; however, controversial reports [66] and important limitations (mainly due to the low number of patients enrolled in the studies) imply the need of larger clinical trials to clarify this issue.
- 2 The expression of the Mas receptor has been documented in mouse and rat corpus cavernosum; however, so far, no study evaluated the expression of this receptor in human erectile tissues, in both physiological and pathophysiological conditions.
- 3 It was reported that Ang II levels are significantly higher in the corpus cavernosum when compared with the plasmatic levels [14]. Moreover, changes in Ang II levels during the different stages of erection and ED patients have been also reported [14]. Thus, in order to understand the physiological role of Ang-(1-7) in the erectile function, it would be valuable to better investigate the levels of this beneficial molecule in both human physiology and pathophysiology.
- 4 Ang-(1-7) has been shown to increase the NO bioavailability within the corpus cavernosum; however, it is still not known whether Ang-(1-7) acts on cavernous nerve terminals or endothelial cell, or both;
- 5 The activation of the Mas receptor evokes notable benefits on erectile tissues [18,73]; however, the underlying intracellular signalling pathways of these actions remain to be clarified.

Conclusion

Both basic research and clinical investigations show that renin–angiotensin-mediated pathways are key regulators of the

erectile function and pathological dysfunction. In particular, the local (instead of systemic) imbalance of this system may contribute to the development of ED. Moreover, the pharmacological blockade of Ang II/AT1-triggered activities might beneficially influence ED in humans. Importantly, the activation of Ang-(1-7)/Mas-mediated pathway might represent a promising target for treating ED.

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Conflict of interest

None to be declared.

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