Angiotensin II receptor antagonists and sympathetic neurotransmission
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CHAPTER 1

Introduction
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Introduction

1. Historical backgrounds

In 1898, Tigersted and Bergman discovered that a rabbit kidney extract, administered intravenously, caused an increase in blood pressure in rabbits. [1]. Their kidney-derived substance was called renin. Goldblatt et al. developed a model of hypertension, which relied on a humoral, kidney-derived agent [2]. Renin itself, however, did not cause vasoconstriction in the isolated perfused dog tail [3]. Two groups, one in the USA (Page et al.) and one in Argentina (Braun-Menendez et al.) showed that renin is actually an enzyme, causing the formation of a pressor peptide. The new peptide was called hypertensin by Braun-Menendez and angiotonin by Page [4,5]. Eighteen years later, both authors agreed on the term angiotensin [6]. In the nineteen-fifties, the amino-acid sequence of angiotensin was elucidated and it became clear that two forms existed: a decapeptide (angiotensin I, Ang I) and an octapeptide (angiotensin II, Ang II), derived from Ang I via removal of the last two amino-acids by a chloride dependent enzyme, now called angiotensin converting enzyme (ACE) [7]. Angiotensin II proved to be a very potent vasoconstrictor, whilst Ang I was more or less inactive [8].

2. Pathways (see fig 1)

Angiotensinogen

This is the major substrate for renin. It is a glycoprotein with a molecular weight of 55-65 kD, depending on its degree of glycolysation [9]. Angiotensinogen is synthesised by the liver. Here, the largest amount of angiotensinogen mRNA can be detected, although lower concentrations have been found in other organs, such as the for instance the brain, vascular smooth muscle, kidney, adrenals, atria and lungs. Apart from being the precursor protein for angiotensin I, angiotensinogen has been reported to be an acute phase-protein [10].
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**Renin**

The molecular weight of the active form of this enzyme is 40 kD. Renin is released as prorenin and activated by removal of a 43-aminoacid segment [11]. Although present in many organs such as the brain, uterus, placenta, adrenal glands and large arteries and veins, the juxtaglomerular apparatus in the kidney is the primary site from which renin is released. Renin release is triggered by one of the following three mechanisms: (1) a fall in blood pressure, registered by baroreceptors in the afferent arteriole, (2) a decreased sodium concentration at the macula densa, (3) stimulation of β-adrenoceptors. The juxtaglomerular apparatus is densely innervated by the sympathetic nervous system. The release of renin is inhibited by Ang II via AT₁-receptors on the juxtaglomerular cells [12].

**Angiotensin I**

The structure of Ang I is found in the N-terminus of angiotensinogen. It is the prohormone of Ang II [8]. Its vasoconstrictor potency is low, but it causes the release of aldosterone from the adrenals [13].
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Angiotensin I Converting Enzyme (ACE)
ACE is a dipeptidyl carboxypeptidase, and a member of the family of zinc metallopeptidases [14]. Its molecular weight ranges from 90-160 kD, depending on its form (endothelial, testicular) and its carbohydrate content. It is bound to the plasma membrane at the C-terminus. It converts Ang I to Ang II by cleaving the C-terminating dipeptide His-Leu. It is the same enzyme as kininase II and therefore also inactivates the vasodilator bradykinin. Additionally, it acts on a range of other peptidergic substrates such as enkephalin, substance P, and luteinizing hormone releasing hormone. The conversion of Ang I into Ang II was originally described to occur within the lung on the pulmonary vascular endothelial surface [15].

Local renin-angiotensin-systems
The classic notion that angiotensinogen is derived from the liver, renin from the kidneys and that Ang I is converted into Ang II in the lung has now been substantially modified and adjusted. All components of the RAS are present in the vasculature [16,17]. ACE is present on the luminal side of endothelial cells throughout the circulation and very significant portions of Ang II are synthesised locally [18,19]. In the human heart 80% of Ang II-formation is due to conversion by a serine proteinase, called chymase [20]. Also in the vasculature, this ACE-independent Ang II formation accounts for the majority of vascular Ang II. In the rat and rabbit, chymase is substantially less active.

Angiotensin II
This octapeptide is the most important effector of the renin-angiotensin-system. For a detailed description see below.

Angiotensin III, IV and angiotensin (1-7)
Angiotensin III and IV are active metabolites of Ang II, formed by subsequent removal of the N-terminal aminoacid by aminopeptidase A. Ang (1-7) is generated by peptidases from Ang I or Ang II [21-23]. A role for the ACE-related enzyme ACE2 in the formation of Ang-(1-7) has been described [24].
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3. AT-receptors

Whitebread et al. [25] and Chiu et al. [26] described two distinct AT-receptor subtypes. These were termed AT$_1$ (sensitive to losartan) and AT$_2$ (sensitive to PD123177) [27].

**AT$_1$-receptor**

In 1991, the AT type 1 receptor was cloned [28], thus revealing that the AT$_1$-receptor is a seven-transmembrane receptor. The receptor protein consists of 359 amino acids and it is G-protein coupled. In rodents and rabbits, two subtypes have been identified, AT$_{1A}$ and AT$_{1B}$, respectively [29-31]. These subtypes share 94% homology. Two distinct genes encode for the AT$_1$-receptor isoforms, which in the rat are located on chromosome 2 and 17 [32]. In contrast to the AT$_{1A}$-subtype, the AT$_{1B}$-receptor can be inhibited by high concentrations (greater than 0.5 µM) of PD 123319 [33,34]. A number of second messenger pathways have been described which are summarised in fig 2. AT$_1$-receptors are mainly found on vascular smooth muscle cells, glomelular mesangial cells in the kidney, in many areas of the brain and in the adrenals. In the heart, the highest density is found in the conducting system [32].

**AT$_2$-receptor**

The AT$_2$-receptor is also a seven transmembrane receptor. It consists of 363 amino-acids, and shares 34% homology with the AT$_1$-receptor [32]. Its gene is located on the X-chromosome. Both G-protein dependent and independent coupling to the second messenger system have been described [32,35]. These phenomena are depicted in figure 3. The AT$_2$-receptor is inhibited in the nanomolar range by PD 123319 [31]. AT$_2$-binding sites are more abundant in embryonic than in adult tissues and is re-expressed after injury and during remodeling [36,37]. Two sub-populations of AT$_2$-receptors, (AT$_{2A}$ and AT$_{2B}$) have been reported to exist [38,39], which display certain differences in binding to PD 123319 and G-protein coupling.

**AT$_3$-receptor**

Ang II binds with high affinity, to this receptor subtype, which is insensitive to both losartan and PD123319 [40]. The receptor was designated ‘AT3’ in the review by Dinh [32]. However, it was not included in the update of angiotensin receptor nomenclature, proposed by the IUPHAR subcommittee on Angiotensin Receptors [31].
Fig. 2. Signal transduction mechanisms and physiological effects mediated by the AT$_1$-receptor. Abbreviations: PLA, phospholipase A; PLC, phospholipase C; JAK, Janus kinase; STAT, signal transducers and activators of transcription; IP3, inositol-1,4,5-triphosphate; DAG, diacylglycerol; PKC, protein kinase C. Adapted from [32], with permission.

**AT$_1$-receptor**

The hexapeptide angiotensin fragment angiotensin IV binds a receptor that is different from the AT$_1$ and AT$_2$-receptor-subtypes [41]. Ang II has a low affinity for this AT-receptor subtype. Its molecular weight is 150-165 kDa and various isoforms may exist [42]. This receptor is widely distributed in different tissues but is mainly found in the brain and kidney.
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Fig. 3. Signal transduction mechanisms and physiological effects mediated by the AT$_2$-receptor. Abbreviations: PLA, phospholipase A; PTP, protein tyrosine phosphatase; PP2A, serine/threonine phosphatase 2A, ERK, extracellular signal-regulated kinase. Adapted from [32], with permission.

4. Physiological role of the renin-angiotensin-sytem

Angiotensin II

Angiotensin II displays a vast array of effects in many different target organs such as the cardiovascular system, adrenals, the brain and kidney. Many different effects have been described and new effects are being discovered continuously. Well-known effects of Ang II are vasoconstriction, the release of aldosterone and catecholamines from the adrenals, vasopressin secretion, positive inotropic and chronotropic effects, calcium mobilisation / phosphoinositide hydrolysis, inhibition of adelylyl cyclase, protein and DNA synthesis, prostaglandin and NO release, superoxide anion production, increased drinking, facilitation of the sympathetic nervous
system (see below), inhibition of renin release and cell growth and proliferation. Virtually all of these effects have all been shown to be mediated by AT₁-receptors [43-45]. The AT₁-receptor seems to play an important role in fetal growth and development [36]. In young rats, the AT₁-receptor is expressed in certain brain areas that play a role in motoric and sensory activity [46]. This AT₁-expression diminishes with increasing age [47]. In adult species, the role of the AT₁-receptor was mostly unknown. However, more and more possible AT₁-mediated effects have been reported recently, such as antigrowth, antihypertrophic and proapoptotic effects [36]. Furthermore, several studies have shown that the AT₁-receptor mediates vasodilation and NO release [48,49].

Pathophysiology

Increasingly, the role of Ang II in various forms of cardiovascular pathology such as for instance diabetic nephropathy, atherosclerosis, neo-intima formation, and remodeling is being studied [17,50,51]. The role of (inhibiting) the RAS in essential hypertension and heart failure will be dealt with in separate paragraphs in this chapter.

Ang III, IV and Angiotensin (1-7)

By acting on AT₁-receptors, Ang III and IV display similar effects as Ang II. The potency of these peptides has been demonstrated to be lower than that of Ang II [52-54]. Recently, effects of Ang IV have been described, mediated by the AT₁-receptor [55,56]. Ang IV plays a role in cognitive function, and in the regulation of cerebral and renal blood flow, as well as in renal Na⁺-handling. Ang IV has antihypertrophic effects and increases plasminogen inhibitor I expression in vascular endothelial cells [42]. Angiotensin (1-7) can counteract many of the Ang II-induced effects and is mostly considered as an endogenous AT₁-receptor antagonist [57]. Indeed, in isolated arteries, Ang (1-7) was shown to antagonise Ang II-induced vasoconstriction [58].
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5. Inhibition of the renin-angiotensin system

Saralasin
One of the first pharmacological tools that became available was saralasin, a peptide analogue of Ang II, in which the amino acids Asp\(^{1}\) and Phe\(^{8}\) were substituted by Sar and Ala [59]. This compound could only be administered intravenously, which made it less suitable for clinical use. Additionally, it displays partial agonistic activity.

Renin inhibitors
Renin being the initiating and rate limiting component of the Ang II forming cascade, it seems logical to inhibit this enzyme for therapeutic purposes. However, thus far, no compound with a clinically useful profile has emerged. Several experimental compounds have been developed, but all of them display very low bioavailability [60].

ACE-inhibitors
The first non-peptide orally administered ACE-inhibitor captopril was designed in 1977 by Ondetti and Cushman [61]. ACE-inhibitors also inhibit the biodegradation of bradykinin. Because bradykinin is a vasodilator, this effect may contribute to the blood pressure lowering actions of ACE-inhibitors. ACE-inhibition with enalapril was shown to reduce morbidity and mortality in congestive heart failure in the CONSENSUS trial [62]. Later, such findings were repeated with other ACE-inhibitors in many other studies. Now, these drugs have firmly established themselves as antihypertensive drugs, and they are the cornerstone in the pharmacological therapy of heart failure. Lastly, ACE-inhibitors can delay renal failure in patients with diabetic nephropathy [63].

AT\(_{1}\)-blockers
The development of non-peptide AT-receptor antagonists started when two Takeda compounds (S-8307 and S-8308) became available [64,65]. These anti-hypertensive compounds were not very potent AT-antagonists, but they were very specific. Chemical modifications by Timmermans et al. ultimately led to the development of the reference compound DuP 753, later called losartan [45]. Since the introduction of losartan, a large number of non-peptide AT\(_{1}\)-receptor antagonists have been developed. The following are currently available for clinical use in the Netherlands: losartan, irbesartan, telmisartan, valsartan, candesartan and eprosartan. They are all registered as antihypertensive drugs. For chemical structures see appendix.
All AT₁-receptor antagonists, except eprosartan, possess a biphenyl component as their core structure. The tetrazolium group, present in losartan, valsartan, irbesartan and embusartan, though not necessary for AT-blocking activity, combines high affinity for the AT₁-receptor with good bioavailability. The imidazole group, originally present in both lead compounds (S-8307 and S-8308) is also present in losartan and eprosartan. In irbesartan it has a cyclopentane substitute and in candesartan and telmisartan it is condensed with a phenyl ring. Valsartan and embusartan do not contain an imidazole group.

Several clinical trials with AT₁-receptor antagonists have been completed. Their results are summarised in table 1. So far, the side-effect profile of AT₁-blockers is the same as that of placebo. The pharmacological profile of the individual compounds is summarised in table 2.
Table 1. Clinical trials with AT<sub>1</sub>-receptor antagonists that have been completed so far.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Drug</th>
<th>Trial</th>
<th>No. of patients</th>
<th>End Points</th>
<th>Global Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>Losartan</td>
<td>LIFE</td>
<td>9193</td>
<td>Mortality, Stroke, Myocardial infarction</td>
<td>Losartan superior to atenolol.</td>
<td>[66]</td>
</tr>
<tr>
<td>With left ventricular hypertrophy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart Failure</td>
<td>Losartan</td>
<td>ELITE I</td>
<td>722</td>
<td>Increase in serum creatinine, All-cause Mortality</td>
<td>Losartan equal to captopril. Fewer adverse effects in losartan group; all cause mortality lower in losartan group.</td>
<td>[67]</td>
</tr>
<tr>
<td></td>
<td>Losartan</td>
<td>ELITE II</td>
<td>3121</td>
<td>All-cause Mortality, CV Mortality</td>
<td>Losartan equal to captopril.</td>
<td>[68]</td>
</tr>
<tr>
<td></td>
<td>Valsartan</td>
<td>Val-Heft</td>
<td>5010</td>
<td>All-cause Mortality</td>
<td>Valsartan superior to ACE-I + diuretic on combined mortality and morbidity.</td>
<td>[69]</td>
</tr>
<tr>
<td>Type II Diabetes</td>
<td>Losartan</td>
<td>RENAAL</td>
<td>1513</td>
<td>Mortality, Creatinine doubling, ESRD</td>
<td>Losartan superior to placebo.</td>
<td>[70]</td>
</tr>
<tr>
<td>With nephropathy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Irbesartan</td>
<td>IDNT</td>
<td>1715</td>
<td>Mortality, Creatinine doubling, ESRD</td>
<td>Irbesartan superior to placebo or amlodipine.</td>
<td>[71]</td>
</tr>
<tr>
<td>In hypertensives</td>
<td>Irbesartan</td>
<td>IRMA II</td>
<td>590</td>
<td>microalbuminuria</td>
<td>Irbesartan superior to placebo.</td>
<td>[72]</td>
</tr>
</tbody>
</table>

Abbreviations: LIFE, losartan intervention for endpoint reduction trial; ELITE, evaluation of losartan in the elderly; Val-Heft, valsartan-heart failure trial; RENAAL, reduction in end-points in non-insulin dependent diabetes mellitus with angiotensin II antagonist losartan; IDNT, irbesartan diabetic nephropathy trial; IRMA, irbesartan microalbuminuria trial; CV, cardiovascular; ESRD, end-stage renal disease; ACE-I, angiotensin I converting enzyme inhibitor.
**Table 2.** Characteristics of the AT₁-receptor antagonists that are currently available in The Netherlands. Reference: [73]

<table>
<thead>
<tr>
<th>Drug (Active Metabolite)</th>
<th>AT₁ receptor Affinity (nmol/l)</th>
<th>Bioavailability (%)</th>
<th>Active Metabolite</th>
<th>Half Life (h)</th>
<th>Protein binding (%)</th>
<th>Dosage (mg/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Losartan (EXP 3174) Cozaar®</td>
<td>IC₅₀ 26.4</td>
<td>33</td>
<td>Yes</td>
<td>2 (6-9)</td>
<td>98.7 (99.8)</td>
<td>50-100</td>
</tr>
<tr>
<td>Valsartan Diovan®</td>
<td>IC₅₀ 2.7</td>
<td>25</td>
<td>No</td>
<td>9</td>
<td>95</td>
<td>80-320</td>
</tr>
<tr>
<td>Irbesartan Aprovel®</td>
<td>IC₅₀ 1.3 – 4.1</td>
<td>70</td>
<td>No</td>
<td>11-15</td>
<td>90-95</td>
<td>150-300</td>
</tr>
<tr>
<td>Candesartan cilexetil (CV11974) Atacand®</td>
<td>K, 0.6</td>
<td>- (42)</td>
<td>Yes</td>
<td>3.5-4 (3-11)</td>
<td>(99.5)</td>
<td>4-32</td>
</tr>
<tr>
<td>Telmisartan Micardis®</td>
<td>K, 3.7</td>
<td>43</td>
<td>No</td>
<td>24</td>
<td>&gt;99</td>
<td>40-80</td>
</tr>
<tr>
<td>Eprosartan Teveten®</td>
<td>IC₅₀ 1.4 – 3.9</td>
<td>15</td>
<td>No</td>
<td>5-7</td>
<td>98</td>
<td>400-800</td>
</tr>
</tbody>
</table>
6. The sympathetic nervous system

Cardiac and circulatory functions are under control of the autonomic nervous system (ANS), divided in the sympathetic nervous system (SNS) and parasympathetic nervous systems (PNS), which globally display opposing functions [74].

A very brief outline, focused on cardiovascular control by the sympathetic nervous system, will be given in this following paragraph [75]:

The afferent neurons of the ANS receive information from baroreceptors, located in the aortic arch, carotids and the heart. Under physiological circumstances, these receptors exercise an inhibition of adrenergic activity and activate parasympathetic stimuli along the vagal route. The actions of the sympathetic nervous system are classically mediated by α- and β- adrenoceptors, which are sensitive to adrenaline, released from the adrenal medulla, and the neurotransmitter noradrenaline, released from sympathetic nerve terminals. At the level of the sympathetic ganglia, another neurotransmitter is used: acetylcholine (ACh), which acts on nicotinic cholinergic receptors. At the peripheral level other (co) neurotransmitters have been identified such as neuropeptide Y and ATP [76].

At the level of the heart and vascular smooth muscle, the following adrenoceptors mediate the effects of adrenergic stimulation:

- β₁-receptors, sensitive to both adrenaline and noradrenaline, are mainly located in the myocardium, the conduction-tissues of the heart, AV-node and the coronary arteries as well as in the kidney. They mediate increases in heart rate and contractil e force, reduction of the refractory period of cardiac muscle, coronary dilatation and renin release.
- β₂-receptors, which are mainly sensitive to adrenaline, are located in the resistance arteries of skeletal muscle and mediate vasodilatation.
- α₁ and α₂-receptors are present on arterial and venous vascular smooth muscle cells. They mediate vasoconstriction, resulting in an increased peripheral resistance and reduced capacitance. Additionally, noradrenaline release is inhibited via presynaptic α₂-receptors on sympathetic nerve terminals.
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7. Interactions between the renin-angiotensin system and the sympathetic nervous system

Since several decades it is well known that the RAS and the SNS can interact at various levels [77-80].

Influence of the SNS on the RAS

Via $\beta_1$-adrenoceptors in the juxtaglomerular apparatus, noradrenaline can provoke the release of renin from the kidney [81]. At the level of the blood vessels, through a $\beta$-adrenoceptor mediated mechanism, both renin and Ang II can be released [82-84].

Influence of the RAS on the SNS

Angiotensin II exerts its influence on the sympathetic nervous system at several levels (fig. 4):

Central Nervous system

In a dog cross circulation study, it was shown that Ang II, infused in the carotid inflow of the head of a dog, which was vascularly isolated but neuronally connected with its trunk, caused an increase in blood pressure in both the donor and in the recipient animal [85]. In many subsequent studies, it was shown that the central administration of Ang II causes an increase in sympathetic outflow and a suppression of baroreflex control [79]. The $\text{AT}_1$-receptor is localised in areas that are involved in blood pressure control such as the nucleus tractus solitarii, and the area postrema, whereas $\text{AT}_1$-receptor blockade can inhibit the effects of centrally applied Ang II [86].

Adrenal Medulla

Angiotensin II stimulates the release of noradrenaline from the adrenal glands [13]. A facilitatory effect of Ang II on stimulation-induced adrenaline release was demonstrated in anaesthetised dogs [87]. However, the doses needed are high and the physiological relevance of these observations remains uncertain. In pithed rats, adrenalectomy failed to alter the responses to Ang II [88] and an infusion of Ang II did not alter plasma adrenaline levels in humans [89].
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Fig. 4. Sites and mechanisms of interaction between the renin-angiotensin system and the sympathetic nervous system. Abbreviations: NA, noradrenaline; A, adrenaline. Adapted from [90], with permission.

Sympathetic ganglia

Injection of Ang II into the blood supply of the caudal cervical ganglia of dogs causes positive chronotropic and inotropic responses. The direct positive inotropic effect of Ang II was achieved at doses that were 60 times higher [91]. In pithed rats, this was shown to be an AT₁-receptor mediated mechanism [92]. Propranolol was demonstrated to inhibit the tachycardic response to Ang II, suggesting a role of the β-receptor [93,94].
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Sympathetic nerve terminals (fig. 5)

In various models, the facilitating effect of Ang II on sympathetic neurotransmission at the peripheral level was demonstrated. This facilitation has been shown to be caused by presynaptic mechanisms, such as an increase of noradrenaline synthesis [95], an increase of stimulation-induced release of noradrenaline [96], and the inhibition of its uptake [97,98]. The enhancing effect of Ang II on stimulation-induced noradrenaline release and vasoconstrictor responses could be antagonised by the peptidergic AT$_1$/AT$_2$-receptor antagonist saralasin [99,100] and by various selective AT$_1$-receptor antagonists such as losartan [101-103], irbesartan [102-104] and eprosartan [105], but not by the AT$_2$-receptor antagonists PD 123177 or PD 123319 [101,103,106]. From these experiments, it is generally concluded that Ang II exerts its effects on sympathetic neurotransmission through the AT$_1$-receptor.

Fig. 5. Facilitatory actions of angiotensin II at the sympathetic nerve terminal and vascular smooth muscle cell. NA: noradrenaline.
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In addition, an increased responsiveness of vascular smooth muscle to NA (postsynaptic facilitation) has been demonstrated [107]. Both the $\alpha_1$ and the $\alpha_2$-adrenoceptor have been shown to be involved [108,109]. In some studies, however, such a postsynaptic facilitation could not be confirmed [110-113]. It is generally believed that the presynaptic facilitation is quantitatively more important than the postsynaptic enhancement.

Studies in isolated tissues have shown that locally produced Ang II can enhance noradrenergic neurotransmission [114,115,116]. Such a local interaction was also demonstrated in the human forearm [84]. These studies indicate a significant involvement of the local RAS in the facilitation of sympathetic neurotransmission.

The mechanism by which Ang II facilitates sympathetic neurotransmission has sparsely been investigated. Ang II, via the $\text{AT}_1$-receptor, activates receptor linked phospholipase C (PLC) resulting in the hydrolysis of phosphatidyl-inositol-4,5-biphosphate (PIP$_2$), producing inositol-1,4,5-triphosphate (IP$_3$) and diacylglycerol (DAG). IP$_3$ releases Ca$^{2+}$ from the sarcoplasmatic reticulum (SR), but this mechanism appears to have little effect on neurotransmission [117]. DAG however, activates protein kinase C (PKC), which through a number of mechanisms plays an important part in transmitter release: (1) by prolonging Ca$^{2+}$ influx, (2) by modulating NA synthesis (through phosphorylation of tyrosine hydroxylase), (3) by modulation of exocytosis (through phosphorylation of exocytotic proteins) [118].

At the post-synaptic level, the protein kinase C pathway was shown to be involved in the facilitation by Ang II of $\alpha$-adrenoceptor mediated responses. [107,119].
8. The sympathetic nervous system and the renin-angiotensin system in hypertension

8.1. The sympathetic nervous system in hypertension

Both the renin-angiotensin system and sympathetic nervous system have been extensively studied in animal models as well as in human hypertensives. Pharmacological tools directed against various components of both blood pressure regulatory systems have been successfully applied in the treatment of hypertension.

The spontaneously hypertensive rat (SHR) has since long been used as a model for human essential hypertension. The substantial role of the sympathetic nervous system in the development and maintenance of hypertension in this model is firmly established. Isolated arteries from SHR display an increased sensitivity to noradrenaline, increased responses to electrical stimulation, whereas the density of noradrenergic nerves is higher [120-122].

Also in humans, although more difficult to demonstrate, the sympathetic nervous system may play a primary role in the pathogenesis of essential hypertension. Plasma NA-levels have proven to be a rather insensitive marker of adrenergic activation [123,124]. However, most studies in subjects with essential hypertension have shown increased sympathetic neuronal activity, as recorded by microneurography [125]. Furthermore, regional NA spillover from the heart and kidneys is increased in essential hypertension [126]. Hypertensive patients respond with greater falls in blood pressure to the administration of propranolol, and with smaller falls in blood pressure to administration of atropine compared to normotensive subjects. These findings have lead to the concept of sympathetic activation and parasympathetic withdrawal in hypertension [127]. This hypothesis has been confirmed in studies in which heart rate variability was assessed using power spectral analysis. In hypertensive subjects, the low frequency element, derived from the cardiac sympathetic nerves, is enhanced. In contrast, the high frequency component, which is indicative for parasympathetic activity, proved to be absent [128].
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8.2. The renin-angiotensin system in hypertension

The efficacy of ACE-inhibitors and AT₁-antagonists in the treatment of hypertension clearly demonstrates the involvement of the renin-angiotensin system in cardiovascular control. However, no strong correlation exists between essential hypertension and various markers of the RAS, such as plasma Ang II or plasma renin [129]. Additionally, blocking the RAS is also effective in patients in which plasma renin is normal or reduced, and even in anephric patients [130]. These observations have led to the concept that the local RAS may be an important contributor to (high) blood pressure. Other mechanisms in which the RAS may be involved in hypertension concern the production of superoxide anions [131]. Through various mechanisms, such as decreased NO-levels and endothelial damage this may lead to vasoconstriction and/or vascular remodeling. In recent years, substantial knowledge has been acquired concerning the role of Ang II in the development of hypertension, hypertensive target organ injury and atherosclerosis through modulation of the OxLDL pathway and its receptors [132]. Essential hypertension is at least partly genetically determined [133]. From the genes encoding the various components of the renin-angiotensin system, the angiotensinogen-gene has proven to be the most interesting candidate; it plays a significant but modest role in human blood pressure variance [134].

8.3. Interaction between the SNS and the RAS in hypertension

The facilitating effect of Ang II on sympathetic neurotransmission was demonstrated to be enhanced in (isolated arteries of) spontaneously hypertensive rats compared to their normotensive controls, the Wistar Kyoto rat [135,136]. Both AT₁-antagonists and ACE-inhibitors can inhibit these effects [111,135]. Recently, Raash et al. demonstrated that chronic ACE-inhibition increased cardiac NA-reuptake in SHR [98]. Interestingly, in patients with essential hypertension, a reduced presynaptic NA-uptake has been described [137]. It is not clear to which extent the sympatho-inhibitory effects of RAS-blockade also apply to humans: Some studies in hypertensives demonstrated that both ACE-inhibition and AT₁-blockade reduce plasma NA [138,139]. In other studies, no such effect was found [140,141]. We already mentioned the limited value of plasma NA-levels. Acute losartan treatment blunted the sympathetic stimulatory effect of cold stress in hypertensive subjects on blood pressure and NA concentrations, without changing baseline plasma NA [142]. On the other hand, chronic ACE-inhibition treatment did not
significantly affect sympathetic nerve traffic, measured by microneurography, nor plasma NA in hypertensive subjects [143].

9. The SNS and the RAS in congestive heart failure

Various forms of congestive heart failure are known to be accompanied by activation of the renin-angiotensin system and the sympathetic nervous system [144]. In the following paragraph, the (mutual interactions between) the RAS and SNS are briefly discussed.

9.1. The sympathetic nervous system in congestive heart failure

In the initial phase of heart failure, the increased sympathetic activity is caused by deactivation of baroreceptors in the aortic arch due to the reduced cardiac output.

In patients with various types of heart failure, a reduced sensitivity of the arterial baroreflex has been demonstrated [145]. This results in a decreased vagal and an increased sympathetic cardiac influence. Plasma levels of noradrenaline have been shown to be increased and heart rate is usually elevated [146,147]. In humans, in studies in which sympathetic nerve traffic was studied with the microneurography technique, the number of sympathetic bursts per minute was higher in patients with heart failure compared to healthy age-matched subjects [148,149]. Probably due to exposure to increased catecholamine-levels, heart failure results in changes in responses to both α- and β-adrenoceptor-stimulation [150]. β1-adrenoceptor density is decreased in the failing heart. Additionally, heart failure leads to (mild) uncoupling of β2-receptors in the myocardium. α1-Adrenoceptor responses are attenuated or unchanged, although the number of receptors is unchanged, possibly as a result of receptor-uncoupling.

The sympathetic activation probably originates from an attempt to compensate for the decrease in cardiac output. In later stages of heart failure, this compensatory mechanism backfires and it is outweighed by the detrimental effects of sympathetic activation such as arrhythmogenesis, increased cardiac workload and oxygen consumption, increased coronary and peripheral vasoconstriction (increased afterload). Lastly, sympathetic stimulation of renin release results in sodium and water retention, ultimately causing congestion. Indeed, sympatho-excitation, reflected by increased levels of plasma NA, is now seen as a risk factor in various, even asymptomatic stages of HF [144,151,152].
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9.2. The renin-angiotensin system in congestive heart failure

As early as 1962, the activation of the RAS in heart failure was reported [153]. In 1978, two groups simultaneously reported that blocking the RAS improves cardiac and systemic haemodynamics [154,155]. It combines afterload reduction with diminished retention of salt and volume. Since then, suppression of the activity/influence of the RAS has shown to have favourable effects in experimental heart failure [156,157] as well as in clinical trials, such as the CONSENSUS-study. For review see [158].

The (deleterious) effects of (locally produced) Ang II in heart failure are now becoming more and more clear and they include fibrosis, cellular growth and remodeling, which mostly involve the AT₁-receptor [159]. AT₁-receptor stimulation appears to mediate opposite effects on myocardial growth and fibrosis [160]. Aldosterone appears to play a role in the myocardial and vascular fibrosis and addition of low dose spironolactone to ACE-inhibitor therapy has been shown to reduce cardiac and overall mortality in severe heart failure [161].

Most studies, in both clinical and experimental heart failure, have demonstrated that myocardial AT₁-receptors are downregulated, while the AT₂-population was shown to be unchanged or up-regulated [159,162]. Selective blocking of the AT₁-receptor has been shown, in a rat myocardial-infarction heart failure model to improve parameters such as left ventricular end-diastolic and end systolic volumes, ejection fraction as well as interstitial collagen deposition and cardiomyocyte size. These effects were counteracted by AT₂-blockade [163]. However, in humans no clear differences between ACE-inhibitors and AT₁-blockers in the treatment of HF have been reported so far. Head-on comparative randomized clinical trials are ongoing.

9.3. Interaction between SNS and RAS in heart failure

In the CONSENSUS trial, ACE-inhibition treatment was associated with a significant reduction of plasma NA-levels [164]. In smaller studies, similar findings were reported with AT₁-blockade by some [165,166] but not by all authors [167]. In addition, a low dose of an ACE inhibitor was shown to increase parasympathetic activity [168].

In a pacing-induced heart failure model, chronic AT₁-blockade was shown to reduce renal sympathetic nerve activity, as well as to enhance baroreflex sensitivity [169]. In analogy to this animal model, similar findings were obtained in humans subjected to chronic ACE-inhibition [148].
10. Synthesis/Conclusions

In this introductory chapter, the history, biochemistry and pathophysiology of the renin-angiotensin system has been outlined. The experimental evidence for its interaction with the sympathetic nervous system has been reviewed. The RAS and the SNS play a pivotal role in the pathophysiology of hypertension and even more so in heart failure. Consequently, there exists important potential for the positive reciprocal feedback-loop between the RAS and the SNS to be involved in the pathophysiology and therapeutic approaches of these conditions.
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References

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Chapter 1


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Introduction


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Chapter 1
Aim of the present investigation

Angiotensin II has a well known facilitatory effect on sympathetic neurotransmission, which is mainly mediated by AT₁-receptors, located on sympathetic nerve terminals. Both the renin-angiotensin system and the sympathetic nervous system are involved in the pathophysiology of hypertension and heart failure. Therefore, this interaction may be a relevant target of pharmacological intervention. ACE-inhibitors and, increasingly, AT₁-receptor antagonists are widely used in the treatment of hypertension, and ACE-inhibitors are a cornerstone in the treatment of heart failure.

Some AT₁-blockers have been shown to inhibit the prejunctional AT₁-receptor. However, very little is known about their sympatho-inhibitory potency. Even less is known about how their prejunctional inhibitory potency relates to their activity regarding inhibition of the AT₁-receptor on vascular smooth muscle cells.

Accordingly, in the present investigation, the sympatho-inhibitory potencies of several AT₁-receptor antagonists were compared. The experiments were performed in vitro in the isolated mesenteric arteries of both rats and rabbits, as well as in vivo in the pithed rat model.

In addition, by investigating the effects of the AT₁-receptor antagonists on dose-response relationships to vasoconstriction caused by Ang II, we established the potency of these compounds regarding inhibition of the AT₁-receptor on vascular smooth muscle.

By combining these two approaches we could compare the ranking order regarding both pre- and postjunctional AT₁-blockade of eprosartan, candesartan, valsartan and embusartan in the pithed rat, and that of eprosartan and candesartan in the isolated rabbit mesenteric artery. Accordingly, the availability of several selective AT₁-receptor antagonists enabled us to pharmacologically distinguish putative receptor subtype differences of the prejunctional and postjunctional AT₁-receptors.

Additionally, we attempted to clarify whether there could be a role of prejunctional AT₂ receptors and AT₁β-receptors by using the unselective AT-receptor antagonist saralasin, and the AT₂-receptor antagonist PD123319, which in high concentrations displays affinity for the AT₁β-receptor subtype.
Aim of the present investigation

In two pathological models, in which both the sympathetic nervous system and the renin-angiotensin system play an important role, we studied the facilitatory role of Ang II as well as its inhibition:

In pithed spontaneously hypertensive rats and their normotensive controls, we compared the sympatho-inhibitory action of the AT₁-receptor antagonist irbesartan.

In the isolated mesenteric artery of rabbits with experimentally induced heart failure as well as in vessels obtained from age-matched control animals, the sympatho-inhibitory action of the AT₁-receptor antagonist eprosartan was studied.

Finally, we investigated the effects of Ang II on sympathetic neurotransmission in humans, using the forearm venous occlusion plethysmography model. With this technique, we studied the effects of Ang II on tyramine-induced vasoconstriction.