Pre- and postsynaptic studies concerning the interaction between the renin angiotensin system and the sympathetic nervous system

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CHAPTER 1

Introduction
Chapter 1

Introduction

1. Historical background

Tigersted and Bergman discovered in 1898 that a rabbit kidney extract, which they called renin, caused an increase in blood pressure in rabbits when administered intravenously. Goldblatt et al. showed convincingly that it was possible to provoke hypertension by means of a humoral, kidney-derived agent. Renin itself, however, showed no vasoconstrictive activity. Renin was reported to catalyse the formation of a pressor-peptide by acting on the plasma protein angiotensinogen by Page et al. and Braun-Menendez et al., and therefore constituted an enzyme. Braun-Menendez named this peptide hypertensin, whereas Page called it angiotonin. In 1958 it was agreed to combine the names and the term angiotensin was introduced. It was till the mid nineteen-fifties before the amino-acid sequence of angiotensin was elucidated. It was discovered that two forms of the peptide existed: a decapeptide (angiotensin I, Ang I) and an octapeptide (angiotensin II, Ang II), which was derived from Ang I via removal of the last two amino-acids by a chloride dependent enzyme, now known as angiotensin converting enzyme (ACE). Angiotensin II was shown to be the more active form and proved a very potent vasoconstrictor. Although the findings of Tigersted and Bergman had no immediate impact, several years later the renin angiotensin system (RAS) was recognised to be of major importance for the homeostasis of blood pressure, blood volume and electrolyte composition.

2. Renin-angiotensin cascade (see figure 1)

2.1 Circulating renin-angiotensin system

2.2.1 Angiotensinogen

Angiotensinogen is synthesised mainly by the liver. It constitutes the major substrate for renin and is a glycoprotein. Its molecular weight varies from 55-65 kD, depending on its degree of glycolysation. Additionally, mRNA of angiotensinogen can be detected in the brain, vascular smooth muscle, kidney, adrenals, atria and lungs.
2.1.2 Renin

The primary site from which renin is released comprises the juxtaglomerular apparatus in the kidney. However, renin is also present in many organs such as the brain, uterus, placenta, adrenal glands and large arteries and veins. It is released as pro-renin and activated by the removal of a 43-aminoacid-segment. Renin has a molecular weight of 40 kD.

Several mechanisms at the level of the kidney are responsible for the release of renin: 1) a decrease in blood pressure, registered by baroreceptors in the afferent renal arteriole, 2) a decreased sodium concentration at the macula densa, 3) stimulation of $\beta_1$-adrenoceptors.

Besides these trigger mechanisms the release of renin is negatively influenced by Ang II. Via stimulation of AT$_1$-receptors on the juxtaglomerular cells the release of renin is inhibited, which may be considered as a negative feedback loop.

2.1.3 Angiotensin I

The structure of Ang I is found in the N-terminus of angiotensinogen. Ang I is the prohormone of Ang II. Its vasoconstrictor potency is low, but it causes the release of aldosterone from the adrenals.

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**Figure 1.** The renin-angiotensin system cascade.
2.1.4 Angiotensin I Converting Enzyme (ACE)

The dipeptidyl carboxypeptidase ACE is a member of the family of zinc metallopeptidases and is bound to the plasma membrane at the C-terminus. Depending on its carbohydrate content and form (endothelial, testicular) the molecular weight ranges from 90-160 kD. ACE is responsible for the conversion of Ang I to Ang II by cleaving the C-terminate dipeptide His-Leu. The pulmonary vascular endothelial surface was originally the site at which the conversion of Ang I into Ang II was reported to occur. Recently, several other (local) sites have been described (see below). Besides acting on Ang I, ACE inactivates the vasodilator peptide bradykinin. Additionally, ACE acts on various other peptidergic substrates such as enkephalin, substance P, and luteinizing hormone releasing hormone.

2.1.5 Angiotensin II

As the primary effector hormone of the RAS this octapeptide causes 2 major effects, that is direct peripheral and renal vasoconstriction and enhanced secretion of aldosterone by the adrenals. Ang II is also known as angiotensin (1-8). A detailed description will follow.

2.1.6 Angiotensin III, IV and angiotensin (1-7)

Degradation products of Ang II are generally referred to as angiotensin fragments. The heptapeptide angiotensin (2-8) (also called Ang III) is derived from Ang II through an enzymatic cleavage of the N-terminal aspartate by aminopeptidase A. The hexapeptide angiotensin (3-8) (also called angiotensin IV) is derived from Ang III by cleavage of the N-terminal arginine by aminopeptidase N. Both Ang III and IV are active metabolites of Ang II. The binding affinity of Ang III for the AT₁- and AT₂-receptor is similar to that of Ang II. However, being more sensitive to enzymatic hydrolysis Ang III is less potent than Ang II. For Ang IV a lower affinity towards the AT₁- and AT₂-receptor compared to Ang II and Ang III has been described. The heptapeptide Ang (1-7) is mainly produced from Ang I by tissue specific endopeptidases. Alternatively, the cleavage of Ang II by a carboxypeptidase acting at the level of C-terminal phenylalanine has been described. Furthermore, the ACE-related enzyme ACE2 appears to be involved in the formation of Ang (1-7).

2.2 Local renin-angiotensin-system

According to classical theories angiotensinogen is synthesised in the liver and renin is released by the kidney, whereas the conversion of Ang I to Ang II is presumed to occur in the lungs. This view has been dramatically revised, owing to intense research activities during the last few years.
Introduction

All the components involved in the enzymatic RAS cascade have been identified in the vasculature\textsuperscript{21,22}. This observation thus suggests that besides a circulating RAS a local RAS is likely to exist. The significance of this local RAS has been described recently\textsuperscript{23-24}.

In addition to ACE, several other enzymes that convert Ang I to Ang II have been identified\textsuperscript{25}. Chymase, a serine proteinase, acts independently from ACE in the formation of Ang II and is responsible for 80\% of the Ang II-formation in the human heart. Additionally, it accounts for the majority of vascular formed Ang II\textsuperscript{26}.

3. Angiotensin II receptors (AT-receptors) and binding sites

The actions of Ang II on various tissues are mediated by binding of the octapeptide to specific cell membrane receptors. Whitebread et al.\textsuperscript{27} and Chiu et al.\textsuperscript{28} were the first to describe pharmacologically two distinct AT-receptor subtypes. These were characterised as AT\textsubscript{1} (high affinity for losartan and low affinity to PD123177) and AT\textsubscript{2} (high affinity for PD123177 and low affinity to losartan)\textsuperscript{29}.

3.1 AT\textsubscript{1}-receptor

Two years after the pharmacological characterisation the AT\textsubscript{1}-receptor was cloned and sequenced in 1991\textsuperscript{30}. These studies identified the receptor protein as a G-protein coupled, seven-transmembrane receptor (7-TMR) that consists of 359 amino acids. A further subdivision into AT\textsubscript{1A} and AT\textsubscript{1B}-receptors has been revealed in rodents and rabbits but not in man\textsuperscript{31,32}. These subtypes share 94\% homology. The genes that encode the two distinct rat AT\textsubscript{1}-receptor isoforms are located on chromosome 2 and 17, respectively\textsuperscript{33}. The signalling pathways of the AT\textsubscript{1}-receptor are now largely understood. They include 'classical' G-protein related cascades leading to PLC-induced stimulation of PKC and of PI-turnover. These and other signalling pathways are summarised in figure 2. The AT\textsubscript{1}-receptor is expressed almost ubiquitously, especially in the cardiovascular system. The AT\textsubscript{1}-receptor subtype is found in the adrenal gland, the vascular smooth muscle, on glomerular mesangial cells in the kidney and in the heart, but also in several areas of the brain.

3.2 AT\textsubscript{2}-receptor

Like the AT\textsubscript{1}-receptor the AT\textsubscript{2}-receptor is a 7-TMR that consists of 363 amino-acids. The nucleic acid homology between the AT\textsubscript{1} and the AT\textsubscript{2}-receptor is only 33-34\%\textsuperscript{34}. Interestingly, the AT\textsubscript{2}-gene is located on the X-chromosome\textsuperscript{35}. Elucidation of the signalling pathways revealed both G-
protein dependent and independent coupling to numerous second messenger systems, see figure 3.3. The AT\(_2\)-receptor is predominantly expressed in fetal tissues\(^{38-40}\). However, under pathological conditions such as congestive heart failure\(^{41}\), renal failure\(^{42}\), after injury and during remodeling\(^{43}\) a dramatic increase of AT\(_2\) expression has been observed\(^{44}\). In analogy to the AT\(_1\)-receptor the AT\(_2\)-receptor is expressed in the adrenal gland and the heart, although it is found predominantly in the uterus, the ovarian granulosa cells, as well as in distinct areas of the brain\(^{45}\). Two sub-populations of the AT\(_2\)-receptor (AT\(_{2A}\) and AT\(_{2B}\), respectively) have been described\(^{46-47}\). Differences in binding to PD123319 and G-protein coupling are reported for the AT\(_{2A}\)- and AT\(_{2B}\)-receptor. The AT\(_2\)-receptor is inhibited by the experimental antagonist PD123319 in the nanomolar range\(^{33}\).

**Figure 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\(^{35}\).

### 3.3 AT\(_2\)-receptor

It has been suggested that a specific AT\(_2\)-receptor would exist\(^{35}\), which is insensitive to both losartan and PD123319, although it displays high affinity for Ang II\(^{48}\). However, the gene encoding for this receptor has not been cloned. It therefore remains a hypothetical entity.
Consequently, it was not included in the update of the angiotensin receptor nomenclature, proposed by the IUPHAR subcommittee on Angiotensin Receptors^{33,49}.

### 3.4 AT\textsubscript{2}-receptor

Ang IV binds to a specific receptor, showing high affinity for the N-terminal domain of Ang (3-8) but a low affinity for Ang I, Ang II and Ang (1-7). This receptor is thus different from the AT\textsubscript{1} and AT\textsubscript{3}-receptor-subtypes\textsuperscript{50}. Its molecular weight is 150-165 kDa and various isoforms may exist\textsuperscript{51}. AT\textsubscript{4}-receptors are widely present in the central nervous system. Additionally they are found in peripheral tissues such as the kidney, adrenals and the heart\textsuperscript{52}.

Figure 3. Signal transduction mechanisms and physiological effects mediated by the AT\textsubscript{2}-receptor. Abbreviations: PLA\textsubscript{2}, phospholipase A; PTP, protein tyrosine phosphatase; PP2A, serine/threonine phosphatase 2A; ERK, extracellular signal-regulated kinase. Adapted from\textsuperscript{35}. 

\[ \text{Ang II} \rightarrow \text{AT}\textsubscript{2} \rightarrow \text{Ca}^{2+} \rightarrow \text{K}^{+} \rightarrow \text{NO} \rightarrow \text{PLA}\textsubscript{2} \rightarrow \text{PTP} \rightarrow \text{Arachidonic Acid} \rightarrow \text{cGMP} \rightarrow \text{G} \rightarrow \text{PP2A, MKP-1, SHP-1} \rightarrow \text{Phosphatases} \rightarrow \text{cGMP} \rightarrow \text{ERK} \rightarrow \text{Growth, Apoptosis, Differentiation, Vasodilatation} \]
4. (Patho)physiological actions of angiotensin and its fragments

4.1 Angiotensin II

The actions of Ang II are numerous and cover a wide area of physiological activities. The cardiovascular system is involved to a major degree. However, other processes and organs are influenced as well by the octapeptide, such as the adrenals, the brain, kidney and DNA synthesis. New effects of Ang II are being discovered continuously.

The 'classic' effects of Ang II are mediated primarily by the AT₁-receptor. These effects entail vasoconstriction, positive inotropic and chronotropic effects, the release of aldosterone and catecholamines from the adrenals, increased drinking, vasopressin secretion, inhibition of renin release, prostaglandin and NO release, calcium mobilisation / phosphoinositide hydrolysis, inhibition of adenylyl cyclase, protein and DNA synthesis, induction of plasminogen activator inhibitor type 1 (PAI-1), superoxide anion production, cell growth and proliferation. Additionally, the facilitation of the effects of the sympathetic nervous system appears to be mediated by the AT₁-receptor and plays a key role in the present investigation.

Concerning the AT₂-receptor there is convincing evidence to support its role in fetal growth and development. However, the AT₂-expression diminishes with increasing age. After birth the ratio of AT₁- versus AT₂-receptor subtype is reversed, the AT₁-receptor being the dominant one in the adult organism. At the level of the vasculature the same phenomenon appears to take place. In the rat aorta the AT₂-receptor expression was reported to be very low in the embryonic state, but rose to high levels in the neonatal period, followed by a rapid decline.

During the past recent years other functions besides fetal growth have been elucidated for the AT₂-receptor. AT₂-receptors were shown to play a role in processes such as cellular growth, differentiation and apoptosis, as well as in vasodilation and anti-thrombosis.

It is evident that Ang II contributes significantly to various types of cardiovascular pathology. Blockade of the renin-angiotensin-system has been proven to be beneficial in the treatment of diabetic nephropathy, atherosclerosis, neo-intima formation, and remodelling of the heart and blood vessels. Concerning two major forms of cardiovascular pathology, that is essential hypertension and heart failure, a detailed paragraph will be included in this chapter.
4.2 Ang III, IV and Angiotensin (1-7)

Ang III is known to act on the central nervous system, leading to an increase in blood pressure, vasopressin release and thirst\[^{65,66}\]. Additionally, it can produce similar effects as Ang II by acting on the AT\(_1\)-receptor and thus induce vasoconstriction, aldosterone release and sodium retention\[^{16}\]. The potency of Ang III is reported to be lower than that of Ang II\[^{67-69}\].

Cardiovascular, mitogenic, renal and fibrinolytic effects have been described for Ang IV\[^{66,70}\]. Additionally, Ang IV appears to be involved in cognitive function\[^{65}\]. In the renal and cerebral vascular bed Ang IV mediates a reduction in vascular resistance by acting on the AT\(_4\)-receptor\[^{52}\]. Additionally, renal handling of Na\(^+\)-ions is mediated by Ang IV. Ang IV has been associated with hypertrophy that appears to be mediated by the AT\(_4\)-receptor\[^{7}\]. Furthermore, Ang IV is known to induce the expression of PAI-1 in vascular endothelial cells\[^{7}\].

Angiotensin (1-7) seems to mediate opposite effects compared to Ang (1-8) and it can therefore be considered as an endogenous AT\(_1\)-receptor antagonist\[^{7}\]. Ang (1-7) was shown to release vasopressin in the central nervous system, mediate diuresis and natriuresis, cause vasodilation, and to provoke anti-angiogenic effects\[^{16}\]. It has been argued that the effects of Ang (1-7) are mediated by specific receptors that differ from the AT\(_1\) and AT\(_2\)-receptor subtype. However, to date no gene-coding for this high affinity receptor has been cloned.

5. Depressants of the renin-angiotensin system

For therapeutic purposes a vast body of research has been invested with the aim to suppress the various activities of the RAS. Accordingly, several agents have become available which can interact with certain components of the RAS and hence impair its activities.

5.1 Renin inhibitors

A promising approach to interfere clinically with the RAS is offered by the inhibition of renin, thereby blocking the initiating and rate-limiting component of the RAS. Consequently, a reduced Ang II plasma level will be observed. Thus far, however, several compounds proved clinically ineffective, probably because of their very low bioavailability\[^{72}\].
5.2 ACE-inhibitors

By selectively inhibiting the angiotensin I-converting enzyme a novel RAS inhibiting therapy was introduced by Ondetti and Cushman in 1977. These so-called ACE-inhibitors act via limiting the biosynthesis of Ang II, the main effector hormone of the RAS. Ondetti and Cushman fulfilled a prerequisite ensuring proper clinical efficacy, by creating a non-peptide, orally administered agent captopril. Besides their direct vasodilator and thus blood pressure lowering capacity, the ACE-inhibitors proved to inhibit the biodegradation of bradykinin. Attenuation of bradykinin degradation, which is a potent vasodilator, may contribute to their blood pressure lowering profile.

The CONSENSUS trial proved that ACE-inhibition (with enalapril) reduced the morbidity and mortality in another major type of cardiovascular pathology, that is congestive heart failure. Many subsequent studies addressing ACE-inhibition in congestive heart failure have largely confirmed the outcome of the CONSENSUS trial. Therefore, ACE-inhibition can be considered as a potent pharmacological antihypertensive treatment, which is also standard treatment of heart failure. Moreover, during the last recent years ACE-inhibition has proven to be beneficial the treatment of certain types of renal disease, especially in patients with diabetic nephropathy.

5.3 AT₁-blockers

A more specific interference with the RAS can be achieved by selectively blocking the receptors through which Ang II mediates its activities. Saralasin, a peptide analogue of Ang II can be considered as the primary pharmacological agent capable of interfering with the RAS. It was synthesised by substituting the aminoacids Asp¹ and Phe² in Ang II by Sar and Ala and it is a combined AT₁/AT₂-receptor antagonist. The clinical use of saralasin is restricted because of its chemical and pharmacological characteristics. Being a peptide, this compound can only be administered intravenously. Additionally, saralasin is a partial agonist.

It was only in the late nineteen eighties that two selective non-peptide AT₁-receptor antagonists (the Takeda compounds S-8307 and S-8308) became available. Originally designed as antihypertensive agents these compounds were not very potent AT-antagonists. However, Timmermans et al. chemically modified them, which resulted in the very potent and highly specific AT₁-receptor antagonist reference compound DuP 753, later called losartan. The success of losartan as a potent and very well tolerated antihypertensive was followed by the introduction of several other non-peptide AT₁-receptor antagonists, such as irbesartan,
Introduction

telmisartan, valsartan, candesartan, embusartan and eprosartan (for chemical structures see Appendix).

The chemical structures of the AT$_1$-receptor antagonists display several similarities. A biphenyl component can be considered as the core structures in all AT$_1$-blockers, except for eprosartan. Additionally, an imidazole group is present in losartan and eprosartan. The tetrazolium group is responsible for the high affinity for the AT$_1$-receptor and good bioavailability, though not a prerequisite for AT-blocking properties. This chemical group is present in losartan, valsartan, irbesartan and embusartan.

Being such promising antihypertensive agents numerous studies, evaluating the clinical profile of the AT$_1$-receptor antagonists, have been conducted the recent years. Additionally, considering the role of the RAS in congestive heart failure, several studies will be completed in the near future regarding this cardiovascular pathology. The results are summarised in table 1.

One particular reason that is partially responsible for the clinical success of the compounds is their side-effect profile. So far, this is the same as that of placebo.

The pharmacological profile of several AT$_1$-receptor antagonists is summarised in table 2.

6. The sympathetic nervous system

The sympathetic nervous system (SNS) will be dealt with concisely, with the emphasis on its cardiovascular control function. It is one of the two components of the autonomic nervous system, the other consisting of the parasympathetic nervous system.

Baroreceptors, located in the aortic arch, carotids and the heart, control both the sympathetic and parasympathetic activity that act antagonistically. Under physiological conditions the sympathetic system is inhibited, whereas the parasympathetic system is activated, mediating its cardiovascular effects via the vagus nerve. A sudden decrease in blood pressure at the level of the baroreceptors will activate the SNS, which becomes dominant as compared to the parasympathetic system. Adrenaline, released from the adrenal medulla and the neurotransmitter noradrenaline, released from sympathetic nerve terminals are the effector compounds of the SNS. They mediate their effects through $\alpha$- and $\beta$-adrenoceptors. At the level of the sympathetic ganglia the neurotransmitter is acetylcholine (ACh), which acts on nicotinic cholinergic receptors.

Sympathetic nervous activity will be followed by activation of the adrenoceptors at the level of the myocardium and the vasculature.
**Table 1.** Major clinical trials with AT₁-receptor antagonists (completed and ongoing)

<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 with LVH</td>
<td>Losartan</td>
<td>LIFE</td>
<td>9194</td>
<td>Mortality, MI, Stroke</td>
<td>Losartan more effective than atenolol. Losartan benefits beyond blood pressure reduction.</td>
<td>82</td>
</tr>
<tr>
<td>elderly hypertensives</td>
<td>Candesartan</td>
<td>SCOPE</td>
<td>4400</td>
<td>CV-mortality, MI, Stroke</td>
<td>Candesartan equal to placebo MI, Stroke superior</td>
<td>81</td>
</tr>
<tr>
<td>with high CV-risk</td>
<td>Valsartan</td>
<td>VALUE</td>
<td>14400</td>
<td>CV-mortality</td>
<td>Trial in progress</td>
<td></td>
</tr>
<tr>
<td>Heart Failure</td>
<td>Losartan</td>
<td>ELITE I</td>
<td>722</td>
<td>Increase in serum creatinine,</td>
<td>Losartan equal to captopril. Fewer adverse effects in losartan group; all cause mortality lower in losartan group.</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>All-cause mortality</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Losartan</td>
<td>ELITE II</td>
<td>3121</td>
<td>All-cause mortality, CV- mortality</td>
<td>Losartan not superior to captopril but better tolerated</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
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</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>86</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Candesartan</td>
<td>CHARM I,II</td>
<td>4000</td>
<td>All-cause mortality</td>
<td>Trial in progress</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Characteristics of the AT₁-receptor antagonists that are currently available in The Netherlands.

<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 (F:EXP 3174)</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>Cozaar®</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valsartan</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>Diovan®</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Irbesartan</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>Aprovel®</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candesartan cilexetil (CV11974)</td>
<td>Kₐ 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>Atacand®</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Telmisartan</td>
<td>Kₐ 3.7</td>
<td>43</td>
<td>No</td>
<td>24</td>
<td>&gt;99</td>
<td>40-80</td>
</tr>
<tr>
<td>Micardis®</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eprosartan</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>
<tr>
<td>Teveten®</td>
<td></td>
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</tbody>
</table>
• \( \alpha_1 \) and \( \alpha_\text{2} \)-receptors are present on arterial and venous vascular smooth muscle cells, mediating vasoconstriction. Stimulation results in an increased peripheral resistance and reduced venous capacitance. Stimulation of prejunctional \( \beta_\text{2} \)-receptors on sympathetic nerve terminals inhibits noradrenaline release.

• \( \beta_1 \)-receptors, when stimulated, mediate cardiac inotropy, chronotropy, AV-conduction as well as a reduction of the refractory period, coronary dilatation and renin release. \( \beta_1 \)-receptors are mainly located in the myocardium, the nodal-tissues of the heart, AV-node, the coronary arteries as well as in the kidney.

• \( \beta_2 \)-receptors are predominantly located in the veins and resistance arteries of skeletal muscle. Their activation causes vasodilatation. Additionally, when stimulated, presynaptic \( \beta_2 \)-adrenoceptors enhance the release of noradrenaline from the nerve endings.

7. The renin-angiotensin system and the sympathetic nervous system

Numerous studies indicate that the RAS and the SNS may interact at various levels within the cardiovascular system. The renin-angiotensin system and the sympathetic nervous system may interact at various levels within the cardiovascular system. 

7.1 Influence of the SNS on the RAS

The vascular \( \beta \)-adrenoceptor has been shown to mediate the release of both renin and Ang II. Additionally, via the renal \( \beta_1 \)-adrenoceptors located in the juxtaglomerular apparatus, renin can be released from the kidney.

7.2 Influence of the RAS on the SNS

The RAS, primarily through Ang II, is known to interact with the SNS at several levels that include the central nervous system, the adrenal medulla, the sympathetic ganglia as well as the sympathetic nerve terminals (figure 4):

7.2.1 Central Nervous system

It has been demonstrated repeatedly in several species that centrally administered Ang II causes an increase sympathetic outflow, whereas the baroreflex control was attenuated in such experiments. The receptor via which Ang II mediates this effect was shown to be the \( \text{AT}_1 \)-receptor, since \( \text{AT}_1 \)-receptor blockade proved to inhibit the effects of centrally applied Ang II. Centrally, the \( \text{AT}_1 \)-receptor appears to be localised in areas such as the nucleus tractus solitarii,
and the area postrema, which are known to play a major role in the central nervous control of blood pressure\textsuperscript{97,98}.

7.2.2 Adrenal Medulla
Ang II is known to stimulate the secretion of the catecholamines (nor)adrenaline by the adrenal medulla\textsuperscript{13}. The Ang II-induced release of adrenaline is probably mediated by the AT\textsubscript{1}-receptor. Losartan proved to inhibit Ang II-induced increase in adrenaline secretion\textsuperscript{99}. A facilitatory action of Ang II on adrenaline release has been described in the anaesthetised dog\textsuperscript{100}. Whether this observation is of physiological relevance is subject to discussion since the doses required are supra-physiological. In humans an infusion of Ang II failed to alter plasma (nor)adrenaline levels\textsuperscript{101}.

![Diagram](image)

**Figure 4.** Sites and mechanisms of interaction between the renin-angiotensin system and the sympathetic nervous system. Abbreviations: NA, noradrenaline; A, adrenaline. Adapted from\textsuperscript{102}.

7.2.3 Sympathetic ganglia
Ang II was shown to mediate positive chronotropic and inotropic responses after infusion into the blood supply of the cervical/stellate ganglia of several species such as dogs and cats\textsuperscript{103,104}. The
effect of Ang II is apparently due to a direct action on the ganglionic cells, because positive chronotropy and inotropy responses persist after chronic denervation of the ganglia. Blockade of the AT₁-receptor abolished the effects evoked by Ang II. Additionally, the β-receptor appears to be involved. Propranolol inhibited the tachycardic response to Ang II.

7.2.4 Sympathetic nerve terminals (figure 5)

It was repeatedly demonstrated that Ang II can enhance the sympathetic neurotransmission at the peripheral level. At the sympathetic nerve terminals Ang II has been shown to block noradrenaline uptake, to enhance noradrenaline synthesis and, most importantly, to facilitate sympathetic transmission by enhancement of noradrenaline release. The receptor via which Ang II mediates these effects is generally assumed to consist of the AT₁-receptor subtype. Ang II-mediated facilitation of sympathetic nerve traffic could be antagonised by the peptidergic AT₁/AT₂-receptor antagonist saralasin and later on by several selective AT₁-receptor antagonists such as losartan, eprosartan and irbesartan. The selective AT₂-receptor antagonists PD123177 and PD123319 were proved repeatedly unable to inhibit Ang II-facilitated sympathetic transmission, thereby excluding a role for the AT₂-receptor.

Figure 5. Facilitatory actions of angiotensin II at the sympathetic nerve terminal and vascular smooth muscle cell. Abbreviations: NA, noradrenaline; VSMC, vascular smooth muscle cell.
An intriguing question was whether the local RAS would be also able to facilitate sympathetic nerve traffic. Indeed, it has repeatedly been shown that locally produced Ang II can enhance noradrenergic neurotransmission in various models\textsuperscript{93,124,125}.

The prejunctional AT\textsubscript{1}-receptor signalling pathway consists of the activation of a receptor linked phospholipase C (PLC). Subsequently, phosphatidyl-inositol-4,5-biphosphate (PIP\textsubscript{2}) is hydrolysed producing inositol-1,4,5-triphosphate (IP\textsubscript{3}) and diacylglycerol (DAG). IP\textsubscript{3}-mediated signalling processes appear not to contribute significantly to the enhancement of sympathetic neurotransmission\textsuperscript{126}. DAG mediated activation of protein kinase C (PKC), however, appears to contribute to the facilitation of transmitter release via several mechanisms; 1) prolongation of Ca\textsuperscript{2+} influx, 2) modulation of NA synthesis (through phosphorylation of tyrosine hydroxylase), and 3) modulation of exocytosis (through phosphorylation of exocytotic proteins)\textsuperscript{127}.

Besides an Ang II-mediated prejunctional facilitation of sympathetic neurotransmission several studies demonstrated an increased responsivenes of vascular smooth muscle to noradrenaline, via both the \(\alpha_1\) and the \(\alpha_2\)-adrenoceptor\textsuperscript{128,129}. This phenomenon is called postjunctional facilitation. Postjunctional facilitation, however, could not be demonstrated in all studies\textsuperscript{130,131}. The prejunctional facilitation is assumed to be more significant compared to the postjunctional enhancement of sympathetic transmission. The AT\textsubscript{1}-receptor signalling pathway involved in postjunctional facilitation of \(\alpha\)-adrenoceptor mediated responses was shown to be dependent on protein kinase C\textsuperscript{128,134}.

8. The renin-angiotensin system and the sympathetic nervous system in hypertension

8.1. The renin-angiotensin system in hypertension

The role of the renin-angiotensin system in hypertension is confirmed by the efficacy of renin-angiotensin blocking agents such as ACE-inhibitors and AT\textsubscript{1}-antagonists Nonetheless, plasma Ang II and/or plasma renin do not correlate strongly with the degree of essential hypertension\textsuperscript{135}. Even more remarkable is the observation that in low renin and anephric hypertensive subjects the administration of ACE-inhibitors can lower blood pressure\textsuperscript{136}. The local RAS may therefore play a crucial role in essential hypertension. Additionally, Ang II may induce oxidative stress, which is presumed to be involved in essential hypertension\textsuperscript{137}. Super oxide anions cause endothelial damage leading to decreased NO-levels. Moreover, via the modulation of the OxLDL pathway
Chapter 1

and its receptors the RAS has been associated with atherosclerosis and the development of hypertension.

On a molecular basis the angiotensinogen-gene has been shown to be involved in human hypertension, which implies that essential hypertension is a genetic disease, at least partly.

8.2 The sympathetic nervous system in hypertension

The efficacy of β-blockers and α-adrenoceptor antagonists in the treatment of hypertension demonstrates indirectly that the sympathetic nervous system is involved in the pathogenesis of hypertension.

The significant role of the sympathetic nervous system in hypertension has been established by means of the model of the spontaneously hypertensive rat (SHR). Studies with isolated arteries of these animals demonstrated an increased responsiveness to electrical stimulation due to the augmented density of noradrenergic nerves. Additionally, an increased sensitivity to noradrenaline has been reported.

Evidence for a primary role of the SNS in the pathogenesis of human essential hypertension was definitely obtained by microneurography studies. These studies in subjects with essential hypertension demonstrated an increased sympathetic neuronal activity. However, the correlation between the plasma noradrenaline-level and the degree of hypertension proved to be poor and therefore plasma noradrenaline cannot be considered as a sensitive marker of sympathetic activation. In essential hypertensive subjects, however, regional measurements of noradrenaline, by means of the sensitive spillover technique, demonstrated increased levels of this catecholamine at synaptic sites.

The responses to (para)sympatholytic treatment in hypertensive subjects has led to the concept of sympathetic activation and parasympathetic withdrawal in hypertension. Hypertensives generally demonstrate a greater drop in blood pressure to propranolol, whereas they respond with a smaller rise in blood pressure to atropine compared to normotensive subjects. Power spectral analysis studies, in which heart rate variability is assessed, have confirmed this hypothesis.

8.3. Interaction between the RAS and the SNS in hypertension

It is evident that the influence of the RAS on the SNS is enhanced in hypertension. In the SHR Ang II-mediated facilitation of sympathetic neurotransmission was demonstrated to be enhanced compared to their normotensive controls. Both AT₁-agonists and ACE-inhibitors were demonstrated to abolish these effects, thus confirming that RAS-induced enhancement of sympathetic transmission is mediated via the AT₁-receptor. Additionally, an Ang II-mediated
Introduction

decrease in noradrenaline-reuptake may contribute to the enhanced facilitation of sympathetic nerve traffic in hypertension. Conversely, chronic ACE-inhibition in SHR increased noradrenaline-reuptake\textsuperscript{152}. In essential hypertensive subjects, a reduced presynaptic NA-uptake has been described\textsuperscript{153}.

Conflicting data exists concerning sympatho-inhibition induced by RAS-blockade in hypertensive humans: Several studies showed that both ACE-inhibition and AT\textsubscript{1}-blockade reduced plasma noradrenaline levels\textsuperscript{154-156}, whereas other did not\textsuperscript{157-159}. However, as reported previously, plasma noradrenaline appears to be an insensitive marker for hypertension.

9. The RAS and the SNS in congestive heart failure

9.1 The renin-angiotensin system in congestive heart failure

The renin-angiotensin system appears to be activated in CHF\textsuperscript{160,164}. Although initially compensatory, persistent RAS-activation displays a deleterious influence on cardiac function. Cellular growth and hypertrophy, remodelling and fibrosis are noxious effects of Ang II that involve the AT\textsubscript{1}-receptor\textsuperscript{161}. On the other hand, AT\textsubscript{2}-receptor stimulation was demonstrated to be beneficial by opposing myocardial growth and fibrosis\textsuperscript{162}. Downstream the RAS-cascade, aldosterone comprises another factor mediating myocardial and vascular fibrosis\textsuperscript{163}.

At the molecular level downregulation of the cardiac AT\textsubscript{1}-receptor in the pathological condition of heart failure has repeatedly been described, whereas the AT\textsubscript{2}-receptor was shown to be up-regulated or unchanged\textsuperscript{161,164-166}.

RAS-blocking therapy by means of ACE-inhibition or AT\textsubscript{1}-receptor antagonism has repeatedly been shown to improve cardiac function, morbidity and mortality in experimental heart failure\textsuperscript{167,168} as well as in congestive heart failure in patients\textsuperscript{169}.

9.2 The sympathetic nervous system in congestive heart failure

Similarly as described for the RAS the SNS appears to be activated in CHF\textsuperscript{160}. A decrease in cardiac output is assumed to form the trigger for sympathetic activation. Furthermore, a decreased sensitivity of the arterial baroreflex can be observed in patients suffering from congestive heart failure\textsuperscript{170}. Consequently, the influence of the activated sympathetic nervous system increases. Plasma noradrenaline levels are reported to be increased and related to the severity of the disease in congestive heart failure\textsuperscript{171-172}. Several microneurography studies have demonstrated that the sympathetic burst activity was higher in patients suffering heart failure.
Chapter 1

compared to healthy age-matched subjects. Heart failure appears to result in a decreased β1-adrenoceptor density and uncoupling of α1- and β1-receptors. These changes are assumed to be caused by the exposure to increased catecholamine-levels.

In early stages of the disease, the activated SNS may contribute to maintain cardiovascular homeostasis and adequate tissue perfusion and is considered to be an adaptive mechanism on short term. On long term, however, activation of the sympathetic nervous system causes detrimental effects on cardiac function, such as a pro-arrhythmogenic activity, increased oxygen consumption and cardiac workload. At the vascular level increased coronary and peripheral vasoconstriction were found. It has become evident that persistent sympathetic activation, reflected by increased plasma noradrenaline levels, is directly related to the prognosis and mortality in CHF.

9.3. Interaction between SNS and RAS in heart failure

The CONSENSUS trial demonstrated that ACE-inhibition treatment was associated with reduced plasma noradrenaline levels. Additionally, selective AT1-receptor blockade by losartan significantly reduced plasma noradrenaline levels in patients with congestive heart failure. RAS-inhibition by chronic AT1-blockade or ACE-inhibition has shown to improve both the baroreflex sensitivity and the sympathetic nerve traffic activity in heart failure.

References

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Introduction


Chapter 1


Introduction


Chapter 1


Chapter 1


Introduction


Chapter 1


Aim of the present investigation

Angiotensin II is known to facilitate sympathetic neurotransmission, primarily via AT$_1$-receptors located on the sympathetic nerve terminals. Additionally, the renin-angiotensin-system (RAS) and the sympathetic nervous system (SNS) are known to be involved in essential hypertension and heart failure. Moreover, a positive feedback loop exists between these two cardiovascular controlling systems. Accordingly, both systems are potential targets in the pharmacological treatment of cardiovascular disease. ACE-inhibitors, AT$_1$-antagonists and β-blockers are considered the drugs of choice in the treatment of hypertension and heart failure.

Although applied widely as therapeutics in hypertension and other diseases little is known concerning the sympatho-inhibitory potency of various AT$_1$-antagonists. Are there differences compared to their ability to inhibit the AT$_1$-receptor at the vascular smooth muscle? And if so, may these differences be explained by differences in affinity or by the existence of various AT$_1$- receptor subtypes? Moreover, is there a difference in the facilitatory effect of angiotensin II in heart failure compared to non-pathological conditions and can this be attenuated by AT$_1$-receptor blockade? Additionally, little is known about the influence of maturation on angiotensin II-mediated facilitation. These various issues were targeted by means of appropriate investigations.

Accordingly, in the present investigation, we studied the validity of a biochemical technique to quantify the actions of the peripheral sympathetic nervous system and its modulation by angiotensin II. This modified approach consisted of a variant of the classical superfusion technique, which involves the use of a radiolabeled tracer ($^3$H) inserted into the neurotransmitter molecule. The experiments were performed in the isolated rabbit thoracic aorta. By means of this technique we studied the sympatho-inhibitory potency of several selective angiotensin AT$_1$-receptor antagonists (telmisartan, losartan, irbesartan, candesartan and eprosartan) on angiotensin II-enhanced sympathetic nerve traffic. This enabled us to characterise these blockers at the prejuncional AT$_1$-receptor. Additionally, we investigated the inhibitory potency of eprosartan and candesartan on angiotensin II-mediated vasoconstriction. The comparison of the inhibitory potency of eprosartan and candesartan at the levels of both prejuncional and postjuncional AT$_1$-receptors enabled us to discriminate pharmacologically between putative receptor subtype differences.
Because of the wide interest in the physiological role of the AT_1-receptor, we investigated the role of the presynaptic AT_1-receptor in angiotensin II-mediated facilitation of sympathetic neurotransmission by means of the AT_1-selective compound PD123319. Additionally, the role of the presynaptic AT_{1B}-receptor was evaluated using PD123319, which in high concentrations demonstrates AT_{1B}-affinity.

In a model of experimentally induced congestive heart failure, a condition where both the SNS and the RAS are known to play a significant role, we investigated the facilitatory role of angiotensin II as well as its inhibition by eprosartan. For comparison, we also studied the postjunctional responses to angiotensin II and their inhibition by eprosartan.

Little is known about the influence of maturation on angiotensin II-mediated responses. We therefore investigated the influence of maturation on the angiotensin II-mediated facilitation of sympathetic nerve traffic as well as on the angiotensin II-mediated vasoconstriction and its inhibition by eprosartan.

Lastly, the validity of our developed model to modulate sympathetic nerve traffic was tested by using fully different cardiovascular drugs, such as various types of voltage dependent calcium channel (VDCC) antagonists.