Chapter 1

Introduction and aim
Heart failure, defining the problem

Heart failure is an ever increasing health problem in Western society. In the Netherlands, almost one in every three individuals aged 55 will eventually develop heart failure.\(^1\) It is a highly debilitating, progressively deteriorating disease, "with a prognosis worse than that of most malignancies".\(^2,3\) The Rotterdam Study shows that after first diagnosis of heart failure, 65% of the patients die within 5 years.\(^1\) Notwithstanding these bleak figures in the past decades enormous advances have been made both in our understanding of the development of heart failure and in its treatment.

Heart failure, in fact, is not one disease, but rather a clinical syndrome with diverse aetiology and many clinical manifestations. The most common causes for heart failure are ischemic heart diseases, including myocardial infarction, and hypertension. Other causes are hereditary cardiomyopathies, valvular malfunctions and congenital aberrations. Signs and symptoms include oedema, fatigue, and shortness of breath. Despite this diversity in causes and consequences, a general model for the development of heart failure can be sketched. Generally speaking, heart failure results when increased demand is requested from viable cardiac tissue over an extended period of time. This increased demand may arise either from a loss of functional myocardium (in the case of myocardial infarction), or from increased pressure (in case of hypertension) or volume (in case of valvular dysfunction). To cope with the sustained, increased demand, local and systemic compensating mechanisms are triggered. To normalise wall tension the heart remodels due to cardiomyocyte hypertrophy and non-myocyte hyperplasia. Systemically, salt and water retention and peripheral vasoconstriction are directed at maintaining filling pressure. However, in time these compensating mechanisms aggravate cardiac function even further. Increased heart rate and cardiac hypertrophy impede cardiac circulation and oxygen supply, whereas systemic vasoconstriction and salt and water retention enhance the volume load on an already compromised heart. Thus, these compensating mechanisms are cause and consequence of their own malicious feed-forward system. In the long run, this mechanism causes a further deterioration of the hemodynamic situation and the eventual development of the syndrome of heart failure.\(^2,4\)

Therapeutic options for the treatment of heart failure have evolved alongside our understanding of the disease. Heart failure has consecutively been described as an oedematous state, and diuretics alleviated these symptoms of congestion. Later when more detailed hemodynamic measurements revealed a loss of cardiac output and increased peripheral vasoconstriction, inotropic drugs and vasodilators were added to the treatment regimes. Despite fairly successful reduction of symptoms, these drugs did not prevent the progression of the disease, or prolong life expectancy. The introduction of Angiotensin Converting Enzyme (ACE) inhibitors was a major breakthrough in the treatment of heart failure. Not only did ACE inhibitors reduce peripheral vasoconstriction and salt and water retention, they also displayed substantial impact on disease progression and survival (reviewed in \(^4,6\))
**Angiotensinogen (1-256)**

**Renin**

**Ang I (1-10)**

**ACE**

**Ang II (1-8)**

**AT\textsubscript{1}**

**Figure 1 | Classical view of the Renin Angiotensin System.** Angiotensin II is cleaved in two enzymatic steps from the 256 amino acids-long angiotensinogen and exerts its effects via the AT\textsubscript{1} receptor. Ang: angiotensin, ACE: angiotensin converting enzyme.

**ACE inhibition in heart failure**

ACE inhibitors are the first widely used inhibitors of the Renin-Angiotensin System (RAS). The RAS is one of the key endocrine systems in blood pressure regulation. The RAS was originally considered a straightforward linear cascade of events occurring in the circulation. Renin, released by the kidney, cleaves a ten amino-acid fragment, Angiotensin (Ang) I, from Angiotensinogen that is produced by the liver. Ang I in itself is considered inactive, but can be cleaved by ACE into Ang II. Ang II activates several receptors, of which the AT\textsubscript{1} receptor is characterised best (Figure 1). Stimulation of the AT\textsubscript{1} receptor has various local and systemic effects aimed at augmenting blood pressure, which comprise of sodium and water retention by the kidney, vasoconstriction, and cardiac hypertrophy.\textsuperscript{7}

Initially, the efficacy of ACE inhibitors in heart failure was attributed exclusively to lowered systemic Ang II concentrations causing vasodilation and hence reducing peripheral resistance and improving cardiac output. It was eventually recognised, however, that the reduction in mortality obtained with ACE inhibitors was largely independent of hemodynamic status and exceeded that of other vasodilators by far.\textsuperscript{8} To some extent, the additional value of ACE inhibitors over classical vasodilators was credited to attenuation of left-ventricular remodelling,\textsuperscript{9,13} but the effect is too modest to provide a full explanation.\textsuperscript{14} Eventually, it was observed that ACE inhibitors reduce the
risk of ‘vascular events’ such as (recurrent) myocardial infarction. Indeed, ACE inhibitors have been shown to improve endothelial function and, in relation to this, prevent thrombosis. Furthermore, long-term ACE inhibitor therapy does not necessarily lower Ang II plasma levels. Feed-back induction of renin activity during ACE inhibitor therapy thus increases Ang I levels subsequently resulting in normal or even elevated Ang II plasma levels. These findings have led to the conclusion that the mechanism of action of ACE inhibitors is less straightforward than anticipated and that in fact the RAS itself is more complicated than previously described.

The RAS revisited: Angiotensin-(1-7)
To date it is recognised that in addition to the circulating RAS, all the RAS components are present locally in many organs, including the heart, kidneys and the vasculature. The components of this local or tissue RAS are derived either from local production or from sequestration from the circulation (reviewed in). Furthermore, more players in the RAS have been recognised, including more metabolising enzymes and more angiotensin peptides that are biologically relevant and can modulate the effects of Ang II. Consequently, our concept of the RAS has evolved from a linear cascade into an intricate network of many metabolites. (Figure 2).

Of these alternative Ang metabolites, Ang-(1-7) has been gaining interest as an endogenous counterplayer of Ang II. Ang-(1-7) can be formed from Ang I via subsequent cleavage by ACE and its recently discovered homologue ACE2, or directly from Ang I by endopeptidases. In fact, thorough in vitro evaluation of the kinetics of angiotensin peptide metabolism by ACE, ACE2, and NEP shows that Ang-(1-7) production is more efficient than Ang II production. Ang-(1-7) has been found to antagonise Ang II at various levels. Firstly, as it has higher affinity for ACE than Ang I, it acts as an endogenous ACE inhibitor. Secondly, Ang-(1-7) antagonises Ang II-induced vasoconstriction in vivo in rat and rabbit aorta and in human vessels, probably by directly inhibiting AT1 signal transduction. Thirdly, Ang-(1-7) has effects of its own that are functionally distinct from Ang II effects. Best studied are its vasodilative and anti-hypertensive properties. Ang-(1-7) is a vasodilator in various species and vascular beds, including rat aorta and mesenteric arteries, porcine coronary and pial arteries, and canine cerebral and coronary arteries. In vivo infusion of Ang-(1-7) increases blood flow in human forearm and in rat kidney, brain, mesentery, and skin. Vasodilatation by Ang-(1-7) is mediated via its own D-Ala7-Ang-(1-7)-sensitive receptor, is endothelium dependent, and involves release of NO, prostaglandins, and/or kinins. In hypertensive rats, Ang-(1-7)-induced blood pressure reduction involves the same mediators.
Introduc
tion and aim

Angiotensinogen (1-256)  
\[ \text{Renin} \]
\[ \text{Ang I (1-10)} \]
\[ \text{Ang-(1-9)} \]
\[ \text{Ang-(1-7)} \]
\[ \text{breakdown} \]
\[ \text{ACE2} \]
\[ \text{ACE} \]
\[ \text{NEP} \]
\[ \text{PEP} \]
\[ \text{Ang II (1-8)} \]
\[ \text{Ang IV (3-8)} \]
\[ \text{Ang III (2-8)} \]

Figure 2 | Updated scheme of the Renin-Angiotensin System. In addition to the scheme sketched in Figure 1, the RAS contains more angiotensine peptides, enzymes and receptors. Our peptide of interest, Ang-(1-7) can be produced via several routes: directly out from Ang I by endopeptidases, or from Ang II by the recently discovered ACE homologue ACE2. ACE2 can also form Ang-(1-9), from which ACE can then form Ang-(1-7), but the affinity of Ang I for ACE2 is substantially lower than of Ang II. Note that Ang-(1-7) itself is also a substrate for ACE.

Studies on the cardiac effects of Ang-(1-7), apart from dilatation of coronary arteries in some species,\textsuperscript{43,44,47} are less abundant. Ang-(1-7) was shown to have cardioprotective effects in ischemia-reperfusion experiments in ex vivo perfused rat hearts.\textsuperscript{52,53} Studies in hearts from genetically engineered rats expressing an Ang-(1-7) producing fusion protein confirm these data.\textsuperscript{54} Furthermore, these rats are protected from isoproterenol-induced cardiac hypertrophy.\textsuperscript{54} In addition to this antihypertrophic effect on cardiomyocytes, Ang-(1-7) inhibits vascular smooth muscle cell proliferation, both in vitro\textsuperscript{55-57} and in vivo in balloon denuded arteries\textsuperscript{58} and after stent implantation.\textsuperscript{59}

The therapeutic relevance of Ang-(1-7) is highlighted by its contribution to the beneficial effects of RAS inhibition. Both systemic and cardiac Ang-(1-7) levels considerably increase during treatment with ACE inhibitors and AT\textsubscript{1} receptor antagonists (AT\textsubscript{1}RA), due to inhibition of Ang-(1-7) breakdown or elevation of its precursor, i.e. Ang II, respectively.\textsuperscript{22,60-62} Antagonising Ang-(1-7) impedes the antihypertensive actions of ACE inhibitors and AT\textsubscript{1}RA.\textsuperscript{63-65} In addition, Ang-(1-7) has also been implicated in the antithrombotic actions of RAS inhibition.\textsuperscript{66,67}
Scope of this thesis

The high incidence of cardiovascular disease eventually leading to heart failure, and the less than optimal effects that can be obtained with modern pharmacotherapeutics warrant the search for new, innovative approaches. Given the profile of Ang-(1-7) as a functional antagonist of Ang II and a vasodilator, its antihypertrophic effect on cardiomyocytes, and its antimitogenic effects on fibroblasts-like cells, Ang-(1-7) could be a novel therapeutic in the treatment of heart failure. Therefore, the first part of this thesis studies the long-term effects of Ang-(1-7) infusion in the development of heart failure after myocardial infarction (Chapter 2) and in reno-vascular hypertension (Chapter 4). In addition, Chapter 3 describes the effect of myocardial infarction on local RAS components that determine cardiac Ang II and Ang-(1-7) concentrations.

To study local cardiac effects of Ang-(1-7) delivery in vivo, systemic infusion is less appropriate. The second part of this thesis therefore describes the development of a gene therapy method with the potential to locally deliver Ang-(1-7) in the heart. Chapter 6 describes the development of the method. In Chapter 7 the method is tested with a well documented gene construct.
References


41. le Tran Y, Forster C. Angiotensin-(1-7) and the rat aorta: modulation by the endothelium. *J Cardiovasc Pharmacol*. 1997;30:676-682.


