Role of Local Renin Angiotensin Systems in Cardiac Damage

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Introduction

The renin angiotensin system (RAS) recently celebrated its 100th anniversary. In 1898, renin was discovered by Tigerstedt and Bergman in rabbit kidney (Tigerstedt and Bergman, 1898). Renin turned out to be the rate limiting enzyme of a proteolytic cascade leading to the formation of angiotensin (Ang) I and Ang II. In this cascade renin splits the liver-derived renin substrate, angiotensinogen (AOGEN), in the plasma to form the decapeptide Ang I (Bader and Ganten, 2000). Ang I is then metabolized further into the octapeptide Ang II via the endothelium-bound angiotensin-converting enzyme (ACE). Ang II is one of the most potent vasopressor substances and releases aldosterone from the adrenal gland. The effects of the peptide are transmitted by two main G-protein-coupled receptors, AT$_1$ and AT$_2$, that were originally defined by the discovery of specific ligands and later confirmed by the cloning of two different genes (Murphy et al., 1991; Sasaki et al., 1991; Kambayashi et al., 1993; Mukoyama et al., 1993).

This was the classic view of the RAS. However, with additional research, it became clear that it is not the whole story. The local generation of angiotensin peptides in different tissues was detected mostly even by locally produced precursors and the concept of a tissue-based RAS emerged (Bader et al., 2001). The most obvious example of such a tissue RAS was found in the brain, where AT$_1$ and AT$_2$ receptors have been located beyond the blood–brain barrier not
accessible for circulating Ang II. However, Ang II can be generated in the brain from locally synthesized AOGEN and renin and activate these receptors playing an important role in vasopressin secretion, and in the control of the baroreflex and the sympathetic output (Bader and Ganten, 2002). Things are not so clear in the heart, where parts of the system are generated locally and others have to be imported from the circulation. However, the functional relevance of a local RAS in the heart in particular for pathophysiological processes of cardiac damage is beyond doubt.

In the heart, Ang I is generated by renin imported from the plasma (van Kesteren et al., 1997; Müller et al., 1998) which interacts with AOGEN partially also derived from the circulation but also locally produced (Campbell and Habener, 1986; Dzau et al., 1987; Hellmann et al., 1988; Lindpaintner et al., 1990; Dostal et al., 1992; Sawa et al., 1992; Sadoshima et al., 1993). Locally synthesized ACE (Zhou et al., 1994; Katwa et al., 1995) then converts Ang I to Ang II (Danser et al., 1992; Neri Serneri et al., 1996; de Lannoy et al., 1998; Müller et al., 1998). The peptide interacts with AT₁ and AT₂ receptors present on cardiac myocytes and fibroblasts (Rogers et al., 1986; Saito et al., 1987; Urata et al., 1989; Rogg et al., 1990, 1996; Sechi et al., 1992; Crabos et al., 1994; Lopez et al., 1994; Matsubara et al., 1994; Regitz-Zagrosek et al., 1995; Booz and Baker, 1996; Haywood et al., 1997; Ohkubo et al., 1997; Wharton et al., 1998). Additionally in the human heart, mast cells contain the enzyme chymase which also metabolizes Ang I to Ang II (Urata et al., 1990, 1994). Circulating as well as locally generated Ang II induces vasoconstriction and exerts direct inotropic (Koch-Weser, 1964) and chronotropic actions on the heart. These effects are enhanced by a facilitation of noradrenaline release from sympathetic nerve endings. In addition, Ang II in the heart induces hypertrophy, inflammation and fibrosis by increasing endothelin, transforming growth factor (TGF)-β, oxidative stress, and cytokines.

An important development was the generation of transgenic and knockout technology in order to study the functionality of local RAS (Stec et al., 1998; Bader et al., 2000). Because polymorphisms in the gene for ACE were linked to the risk for cardiac diseases (Cambien et al., 1992; Schunkert, 1997) and ACE inhibitors as well as AT₁ receptor antagonists were extremely effective drugs for the treatment of heart failure (CONSENSUS Trial Study Group, 1987; Pfeffer et al., 1988, 1992; Sharpe et al., 1988, 1991; SOLVD, 1992; Swedberg et al., 1992; Pitt et al., 1997, 2000; Yusuf et al., 2000) numerous transgenic models have been generated to study the role of the RAS in cardiac damage. This chapter will summarize the pathophysiological functions of the cardiac RAS.
AT₁ and cardiac hypertrophy

Hypertrophy and fibrosis are the most important pathophysiological effects induced by Ang II in the heart. The growth-promoting actions of the peptide were originally discovered in adrenal cells and fibroblasts (Schelling et al., 1991) where Ang II elicits DNA synthesis and proliferation. There is ample evidence that locally produced Ang II is also involved in the induction of cardiomyocyte growth and cardiac hypertrophy. ACE inhibitors and AT₁ antagonists prevent or decrease cardiac hypertrophy in humans (Nakashima et al., 1984; Kaplan, 1985) and reduce or cause regression of left ventricular hypertrophy in experimental aortic banding (Kromer and Riegger, 1988), even at doses that do not reduce blood pressure (Linz et al., 1989). Accordingly, other models of left ventricular hypertrophy like SHR, TGR(mREN2)27 (Mullins et al., 1990), or isoproterenol-infused rats respond readily to these drugs with a reduction in left ventricular weight accompanied by a decreased myocardial fibrosis (Sen et al., 1980; Brilla et al., 1991; Nagano et al., 1991, 1992; Pahor et al., 1991; Böhm et al., 1996). In some models, these changes were not correlated with blood pressure reduction or plasma Ang II levels, but instead with a reduction in cardiac Ang II concentrations (Nagano et al., 1991, 1992; Böhm et al., 1996).

The possible implication of the cardiac RAS has also been studied in the remodelling of the myocardium after myocardial infarction which for the most part is a hypertrophic process (Pfeffer, 1995). Some authors have reported transiently increased AOGEN mRNA in myocardial infarction and failure (Drexler et al., 1989). In chronic heart failure induced by ligation of the left coronary artery in rats an enhancement of ACE mRNA was found, which correlated with the cardiac ACE activity, but not with ACE activity in other organs (Hirsch et al., 1991).

To elicit cardiac hypertrophy, Ang II interacts with AT₁ receptors, which exerts its effects via several intracellular signalling pathways mediated by G proteins (Dostal, 2000; Eguchi and Inagami, 2000; Ruwhof and van der Laarse, 2000). Besides intracellular calcium surges and protein kinase C activation, small GTP-binding proteins like RAS and RhoA as well as tyrosine kinase cascades are activated including several members of the mitogen-activated protein (MAP) kinase family and the Jak/STAT pathway. Finally, transcription factors like AP₁ and

with a particular focus on the findings derived from transgenic animal models.
the STATs are activated which initiate the expression of growth related
genes. Moreover, the phosphorylation of the ribosomal protein S6 and
thereby protein synthesis is increased.

Several cofactors have been implicated in the effects of Ang II on
cardiomyocyte growth. Endothelin has been shown to be released by
stretch and Ang II in the heart and in some models endothelin receptor
antagonists block cardiac hypertrophy induced by Ang II (Ito et al.,
1994; Arai et al., 1995; Yamazaki et al., 1996). The source of endothelin
maybe the cardiac fibroblasts which also generate other growth factors
when activated by Ang II such as TGF-β and fibroblast growth factor-2
(Kim et al., 1995; Gray et al., 1998; Pellieux et al., 2001). For these two
factors a crucial involvement in Ang II-induced cardiac hypertrophy
has been shown using specific knockout mouse models (Pellieux et al.,
2001; Schultz et al., 2002).

Another possible mediator of Ang II-induced cardiac hypertrophy
may be norepinephrine released by Ang II from sympathetic nerve end-
ingings in the heart. Norepinephrine was shown to trigger hypertrophy and
in a positive feedback loop also RAS activation via α- and β-adrenergic
receptors (Bogoyevitch et al., 1996; Yamazaki and Yazaki, 2000).

In addition, reactive oxygen species are generated by Ang II via the
AT₁ receptor by the activation of NADPH oxidases in the heart. These
free oxygen radicals are important for several signaling pathways in-
cluding the transactivation of the epidermal growth factor (EGF) recep-
tor by the AT₁ receptor (Griendling and Ushio-Fukai, 2000). It has been
shown that in cardiac fibroblasts and vascular smooth muscle cells
EGF-receptor transactivation is essential for the hypertrophic actions
mediated by the AT₁ receptor (Eguchi and Inagami, 2000; Griendling
and Ushio-Fukai, 2000). The relevance of reactive oxygen species was
demonstrated using mice lacking a subunit of NADPH oxidase (Bendall
et al., 2002). These animals were resistant against Ang II-induced car-
diac hypertrophy.

Another important factor involved in cardiac hypertrophy is me-
chanical stretch of the cardiomyocytes. There is evidence that mechanical
stretch induces Ang II generation and that this effect is crucial for
the development of cardiac hypertrophy (Dostal, 2000). Stretch induces
the release of Ang II in the myocardium as well as from cardiomyocytes
in culture (Sadoshima et al., 1993; Leri et al., 1998). Ang II in turn in-
creases the expression of RAS components such as AOGEn, ACE, AT₁,
and AT₂ in a positive feedback loop (Kijima et al., 1996; Tamura et al.,
1998; Malhotra et al., 1999). AOGEn and AT₁ are induced by p53 bind-
ing to the respective promoter regions after activation by the AT₁
receptor (Leri et al., 1998). AOGEn is additionally induced by the same
receptor involving the Jak/STAT pathway of transcription factors, which was already mentioned above, to induce growth (Mascareno et al., 1998).

Transgenic animal models have been generated to solve the question whether mechanical stretch or whether the cardiac RAS alone are able to induce cardiac hypertrophy independently. Mice lacking AOGs (own unpublished results) or AT$_1$ receptors (Hamawaki et al., 1998; Harada et al., 1998) develop cardiac hypertrophy after volume or pressure overload, respectively. Cardiomyocytes isolated from AOG knockout mice respond to mechanical stretch by activating MAP kinas as do control cells. However, in contrast to control cells this effect is not blocked by AT$_1$ antagonists (Nyui et al., 1997). These results indicate that there are redundant pathways of growth induction by stretch in cardiomyocytes circumventing the RAS. However, the default mechanisms involve Ang II and the AT$_1$ receptor.

Transgenic experiments designed to solve the opposite question, whether or not Ang II alone is able to induce cardiac hypertrophy without mechanical stretch gave controversial results. Transgenic rats overexpressing ACE predominantly in the heart have been produced (Tian et al., 1996). Despite very high cardiac levels of cardiac ACE activity, there were no morphological alterations unless the heart was pressure overloaded with aortic banding. This treatment resulted in a significantly higher hypertrophic response in the ACE-transgenic rats than in control animals. The results support the important role of Ang II in stretch-induced hypertrophy but deny an autonomous effect. In contrast, mice expressing AOG exclusively in the heart remained normotensive but nevertheless developed cardiac hypertrophy (Mazzolai et al., 1998). This finding indicates that the local formation of Ang II induces cardiac damage independent of blood pressure elevation. The importance of local angiotensin generation in end organs was confirmed in a hybrid mouse model carrying a rat AOG transgene on a knockout background (Kang et al., 2002). These animals developed hypertension due to the exclusive expression of AOG in liver and brain. However, since local AOG synthesis in kidney and heart was absent, cardiac hypertrophy and renal fibrosis was attenuated in these mice.

Transgenic animal models have been reported that overexpress AT$_1$-receptors in the heart by the use of the $\alpha$-myosin-heavy chain promoter (Hein et al., 1997; Paradis et al., 2000; Hoffmann et al., 2001). However, the phenotypes of the transgenic animals generated were dramatically different. The mouse models exhibited a drastic cardiac hypertrophy and the animals died after several days (Hein et al., 1997) to weeks
(Paradis *et al.*, 2000) of age. In sharp contrast, the rats appeared absolutely normal unless the heart was pressure-overloaded by aortic banding (Hoffmann *et al.*, 2001). With this treatment, the AT<sub>1</sub> receptor transgenic rats also exhibited increased hypertrophy compared to controls, similar to the observations in the ACE transgenic rats. The difference may be related to a species-specific sensitivity of mouse and rat hearts for Ang II-related effects. However a transgenic mouse model was generated in which Ang II is produced exclusively in the heart and is independent of any other RAS component (van Kats *et al.*, 2001). The investigators used a unique artificially engineered protein (Methot *et al.*, 1997). The animals show dramatically enhanced Ang II levels in the heart but no cardiac hypertrophy. Thus, the issue whether or not Ang II alone can induce cardiac hypertrophy remains controversial. Mechanical stretch employs the local cardiac RAS for growth promotion, but can also use alternative pathways.

**AT<sub>1</sub> and cardiac fibrosis**

Concomitantly to hypertrophy, most stimuli also induce cardiac fibrosis namely the proliferation of cardiac fibroblasts and the excessive deposition of extracellular matrix in the cardiac interstitium (Booz and Baker, 1995). The resulting increase in stiffness causes ventricular dysfunction and finally heart failure mostly through diastolic dysfunction. Therefore, fibrosis is of major pathophysiological relevance. Ang II is directly involved in the development of cardiac fibrosis (Booz and Baker, 1995). Chronic Ang II infusion induces fibrosis and ACE inhibitors as well as AT<sub>1</sub> receptor antagonists can ameliorate fibrosis induced by pressure overload. Activation of the AT<sub>1</sub> receptor in fibroblasts again activates the MAP kinases and the Jak/STAT pathway which induce expression of angiotensinogen and fibrosis-related proteins such as collagens as well as cell proliferation (Booz and Baker, 1995; Murasawa *et al.*, 2000).

A recent experiment employing chimeric mice that carry cardiac cells without AT<sub>1</sub> receptors surrounded by normal tissue has shown that the activation of fibroblasts by Ang II depends on the interaction of the peptide with neighbouring cardiomyocytes (Matsusaka *et al.*, 1999). This observation indicates that cardiomyocytes release a paracrine factor after Ang II stimulation, possibly TGF-β, which is mitogenic for fibroblasts (Lee *et al.*, 1995).

Endothelin may also play role in this conversation between cardiac myocytes and fibroblasts. It is released by cardiomyocytes after Ang II stimulation and activates collagen synthesis in fibroblasts (Guarda *et al.*, 1997).
1993; Rossi et al., 1999). This effect is direct but may be enhanced by stimulation of local TGF-β release (Belloni et al., 1996; Gandhi et al., 2000). Accordingly, two transgenic rat models with hypertension and marked end organ damage support the role of endothelin in Ang II-induced cardiac fibrosis. In transgenic rats, TGR(mREN2)27, carrying the mouse renin gene, ren-2 (Mullins et al., 1990), it was shown that cardiac fibrosis can be blunted by blockade with a combined ETA/ETB-endothelin receptor antagonist (Seccia et al., 2003). Furthermore, inhibition of the endothelin-converting enzyme in double transgenic rats expressing both, the human renin and angiotensinogen genes (Ganten et al., 1992), also attenuates the upregulated cardiac collagen synthesis (Müller et al., 2002). Another possible mediator of cardiac fibrosis in the double transgenic rat model is connective tissue growth factor, which may act via stimulation of TGF-β release (Finckenberg et al., 2003). The same rat model has also shed light on aldosterone as mediator of Ang II-induced fibrosis, since when these rats are treated with the mineralocorticoid receptor antagonist spironolactone, cardiac fibrosis is attenuated (Fiebeler et al., 2001). Aldosterone may facilitate Ang II signaling by upregulating AT1 receptors (Sun and Weber, 1993; Robert et al., 1999) or the signaling of mediators of Ang II action such as EGF (Krug et al., 2002). Finally, reactive oxygen species seem to play a role in Ang II-induced cardiac fibrosis since mice lacking NADPH oxidase do not develop fibrosis (Bendall et al., 2002).

However, the issue of a crucial cardiomyocyte-derived mediator of the profibrotic actions of Ang II on fibroblasts such as endothelins and/or TGF-β remains controversial since growth-promoting actions of Ang II on pure cardiac fibroblasts in culture have been repeatedly demonstrated (Booz and Baker, 1995).

**AT₂ in cardiac hypertrophy and fibrosis**

Surprisingly, a recent study using AT₂-knockout mice has shown that this receptor is essential for cardiac hypertrophy induction by pressure overload (Senbonmatsu et al., 2000; Ichihara et al., 2001). This effect may be mediated by a reduced phosphorylation of the ribosomal protein S6. These results contradict earlier findings employing AT₂ antagonists, which showed antigrowth effects exerted by the AT₂ receptor (Booz and Baker, 1996). Furthermore, another strain of AT₂-deficient mice did not show any difference in hypertrophy development after pressure overload and vascular hypertrophy was even enhanced in these mice (Brede et al., 2001; Wu et al., 2002). Accordingly, a transgenic
mouse overexpressing the AT$_2$ receptor in the heart was less susceptible to AT$_1$-mediated actions compared to controls (Masaki et al., 1998) and developed less fibrosis after Ang II infusion (Kurisu et al., 2003), whereas hypertrophy induction was equal (Sugino et al., 2001). Ang II-induced fibrosis is reduced in these animals by a mechanism involving the kallikrein kinin system (Kurisu et al., 2003), which has been shown to have antihypertrophic and antifibrotic actions in the heart by the use of tissue-kallikrein overexpressing transgenic rats (Silva et al., 2000).

After myocardial infarction, remodelling of the myocardium is part of the wound healing process implicating fibrosis and cardiomyocyte hypertrophy. In the AT$_2$ receptor overexpressing mice this remodelling is facilitated (Yang et al., 2002) and in AT$_2$-deficient animals it is blunted, development of heart failure is exacerbated (Adachi et al., 2003) and the heart may even rupture after infarction (Ichihara et al., 2002). Thus, the majority of data support an antihypertrophic and antifibrotic action of the AT$_2$ receptor but the issue still needs clarification (Inagami and Senbonmatsu, 2001).

**Conclusion**

Locally generated Ang II has numerous effects on the heart with significant pathophysiological impact. Cardiac hypertrophy is induced either by a direct action on cardiomyocytes in concert with mechanical stretch or by the release of mediators such as endothelin, TGF-$\beta$ and reactive oxygen species from cardiac fibroblasts. This crosstalk between myocytes and fibroblasts in the heart is also of major importance for the Ang II-induced cardiac fibrosis which also implicates TGF-$\beta$ and endothelin as well as aldosterone.

Cell type-specific transgenic and knockout animal models will help to clarify the pathophysiological relevant communication between cardiac myocytes and fibroblasts and the relative importance of the two angiotensin receptors, AT$_1$ and AT$_2$ in these processes.

**References**


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