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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

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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 AT_1 and AT_2 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 AT_1 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

with a particular focus on the findings derived from transgenic animal models.

AT_1 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 AT_1 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 AT_1 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 AP_1 and

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 endings 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 AT_1 receptor by the activation of NADPH oxidases in the heart. These free oxygen radicals are important for several signaling pathways including the transactivation of the epidermal growth factor (EGF) receptor by the AT_1 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 AT_1 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 cardiac hypertrophy.

Another important factor involved in cardiac hypertrophy is mechanical 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 increases the expression of RAS components such as AOGEN, ACE, AT_1, and AT_2 in a positive feedback loop (Kijima *et al.*, 1996; Tamura *et al.*, 1998; Malhotra *et al.*, 1999). AOGEN and AT_1 are induced by p53 binding to the respective promoter regions after activation by the AT_1 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 AOGEN (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 AOGEN knockout mice respond to mechanical stretch by activating MAP kinases 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 AOGEN 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 AOGEN transgene on a knockout background (Kang *et al.*, 2002). These animals developed hypertension due to the exclusive expression of AOGEN in liver and brain. However, since local AOGEN 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 α-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_1 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_1 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_1 receptor antagonists can ameliorate fibrosis induced by pressure overload. Activation of the AT_1 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_1 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.*,

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 ET_A/ET_B-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 AT_1 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).

AT_2 in cardiac hypertrophy and fibrosis

Surprisingly, a recent study using AT_2-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 AT_2 antagonists, which showed antigrowth effects exerted by the AT_2 receptor (Booz and Baker, 1996). Furthermore, another strain of AT_2-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 exarcerbated (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-β 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-β and endothelin as well as aldosterone.

Cell type-specific transgenic and knockout animal models will help to clarify the pathophysiologically relevant communication between cardiac myocytes and fibroblasts and the relative importance of the two angiotensin receptors, AT_1 and AT_2 in these processes.

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