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Angiotensin deficiency in mice leads to dilated cardiomyopathy

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Abstract

To explore the role of angiotensin II, we assessed hemodynamics and cardiac function in angiotensinogen-deficient mice in comparison to wild-type animals. Left ventricular end-diastolic diameter and wall thickness were evaluated by echocardiography and systolic and diastolic left ventricular function by pressure–volume relations using a micro-conductance catheter. Compared to wild-type animals, the angiotensinogen-deficient mice were hypotensive and showed impaired systolic function. The hearts were dilated, demonstrated by echocardiography and by a right-ward shift of the pressure–volume loops, but end-diastolic pressure, isovolumic relaxation (τ) and diastolic stiffness were unchanged. Afterload, however, was reduced leading to maintained cardiac output. Although a blockade of the renin-angiotensin system via angiotensin converting enzyme inhibitors or angiotensin AT_1 receptor antagonist is beneficial after cardiac failure, the absence of angiotensin peptides during the ontogenesis leads to dilated cardiomyopathy.

Keywords: Angiotensinogen-deficient mice; Renin angiotensin system; Cardiac function; Cardiomyopathy; Pressure–volume relation

1. Introduction

Angiotensin II is a potent bioactive peptide formed by the enzymatic actions of renin and angiotensin converting enzyme on angiotensinogen and angiotensin I, respectively. Like angiotensin receptors AT_1 and AT_2, angiotensin II is widely —-distributed in the mammalian heart and plays an important role in the cardiovascular homeostasis (Baker et al., 1992). It is released from the myocardium during myocardial ischemia (Sutton and Sharpe, 2000) and contributes to the impact of ischemic damage (Paul et al., 1995). It was also shown that an increase of cardiac angiotensin II by injection or by over-expression of components of the renin-angiotensin system in transgenic animals leads to cardiovascular abnormalities like hypertrophy and hypertension (Hein, 1999). Both effects can be inhibited by competitive antagonists of the AT_1-receptor subtype, indicating that angiotensin AT_1 receptors mediate these effects (Unger et al., 1998). In addition, Ichihara et al. (2001) could demonstrate the importance of the angiotensin AT_2 receptor in hypertension-induced fibrosis.

Tanimoto et al. (1994) generated a mouse lacking angiotensinogen and angiotensin II by gene-targeting technique. The animals are characterized by increased infant death, histopathological renal abnormalities, a marked hypotension and an over-expression of the renin gene (Tanimoto et al., 1994; Kim et al., 1995; Niimura et al., 1995).

We studied these angiotensinogen-deficient mice to investigate whether the lack of angiotensin II influences cardiac performance. Since both cardiac and peripheral effects were anticipated, either directly or secondary as compensatory mechanisms, our study was performed in intact animals. Cardiac dimensions were assessed by echocardiography and cardiac function by left ventricular pressure–volume relations obtained by miniature pressure–conductance catheter.

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2. Materials and methods

2.1. Animals

Mice lacking angiotensinogen were generated by Tanimoto et al. (1994). Male mice with two disrupted angiotensinogen-alleles and wild-type mice (each group: $n=8$), harboring the same genetic background were used for the studies at an age of 11 months. Mice were kept under standardized conditions with an artificial 12-h dark–light cycle. Experiments were carried out according to the National Institutes of Health guidelines for the care and use of laboratory animals and were approved by the local authorities.

2.2. Echocardiography

The animals were anaesthetized by ketamine (10 μg/g, i.p.) and xylazine 2% (5 μg/g, i.p.) and imaged in the parasternal short-axis plane through the anterior chest using the VINGMED echocardiography system FIVE (7.5 MHz, Horten, Norway). Electrocardiographic clips were placed on the limbs for recording heart rate and timing of intracardiac events. After two-dimensional imaging, a single M-mode line was directed across the midventricular short-axis view perpendicular to the anterior and posterior walls. Position was verified using the two-dimensional image updated every few seconds. Data were analyzed using leading-edge conventions (Feldman et al., 2000).

2.3. Surgical procedures and hemodynamic measurements

The animals were anaesthetized with thiopental (125 μg/g, i.p.), intubated and artificially ventilated (200 strokes/min; tidal volume 8 μl/g bodyweight; FiO_2 21%). For continuous registration of LV pressure–volume (PV) loops (Feldman et al., 2000; Georgakopoulos et al., 1998) a 1.4 F micro-conductance–pressure catheter (ARIA SPR-719; Millar Instruments, Houston, TX, USA) was positioned in the left ventricle via the right carotid artery. Calibration of the recorded volume signal was obtained by the hypertonic saline (10%) wash-in technique (Steendijk et al., 1998). All measurements were performed while ventilation was turned off momentarily.

Indices of systolic and diastolic cardiac performance were derived from ventricular pressure–volume data obtained both at steady state and during transient preload reduction induced by direct occlusion of the abdominal vena cava inferior. Cardiac preload was indexed as the left ventricular end-diastolic volume (LVEDV) and end-diastolic pressure (LVEDP). Cardiac afterload (E_a) was defined as left ventricular pressure (LVP)/ stroke volume. Myocardial contractility was quantified by the peak rate of rise in left ventricular pressure (dP/dt_{max}) and by the slope (SdP) of the relation between dP/dt_{max} and left ventricular end-diastolic volume. In addition, we determined the slope (end-systolic elastance, E_{es}) and intercept (end-systolic volume at 70 mm Hg, V_{70}) of the end-systolic pressure–volume relationship (ESPVR) (Steendijk and Baan, 2000).

Diastolic performance was measured by peak dP/dt_{min}, the time constant of isovolumic pressure relaxation (τ) (Feldman et al., 2000; Georgakopoulos et al., 1998) and by the diastolic stiffness constant b, determined from a monoexponential fit to the end-diastolic pressure–volume points.

Furthermore, left ventricular end-systolic volume (LVESV), cardiac output and ejection fraction were determined by customized software (IOX V 1.5, Emka, France).

2.4. Statistical analysis of data

All data are expressed as means ± S.E.M. and were analyzed by Student's t-test. P values <0.05 were accepted as significant.

3. Results

3.1. Echocardiography

M-mode analysis showed a significantly increase in left ventricle chamber diameter in the angiotensinogen-deficient

Table 1
Characterization of hemodynamic parameters in angiotensin-deficient animals and their wild-type controls

	Control	Knockout	$P<$
SBP (mm Hg)	90.0 ± 3.0	60.0 ± 3.1	0.0001
HR (bpm)	400 ± 14	416 ± 28	n.s.
CO (ml/min)	10.9 ± 1.9	12.8 ± 0.5	n.s.
LVEDV (μl)	68.0 ± 6.7	110.2 ± 13.1	0.005
LVESV (μl)	44.8 ± 3.2	78 ± 12.8	0.01
EF (%)	39.7 ± 2.7	27.3 ± 2.9	0.01
LVP_{max} (mm Hg)	98.1 ± 3.4	62.7 ± 3.5	0.001
dP/dt_{max} (mm Hg/s)	6167 ± 350	3349 ± 314	0.001
SdP (mm Hg/s/μl)	89 ± 11	56 ± 9	0.05
E_{es} (mm Hg/μl)	2.09 ± 0.45	1.45 ± 0.15	n.s.
V_{70} (μl)	35 ± 3	70 ± 5	0.001
E_a (mm Hg/μl)	3.9 ± 0.6	1.8 ± 0.2	0.001
dP/dt_{min} (mm Hg/s)	− 5827 ± 270	− 3677 ± 347	0.01
τ (ms)	10.5 ± 0.6	10.6 ± 0.3	n.s.
LVEDP (mm Hg)	8.5 ± 1.9	7.5 ± 1.5	n.s.
b (μl^{-1})	0.12 ± 0.03	0.1 ± 0.06	n.s.

Characterization of hemodynamic parameters in angiotensin-deficient animals (knockout) and their wild-type controls (control) (each group: $n=8$, n.s. = not significant). SBP: systolic blood pressure, HR: heart rate, CO: cardiac output, LVEDV and LVESV: LV end-diastolic and end-systolic volume, EF: ejection fraction, LVP: left ventricular pressure, dP/dt_{max} and dP/dt_{min}: peak rate of LVP increase and decrease, SdP: slope of relation between dP/dt_{max} and left ventricular end-diastolic volume, LVEDP: E_{es}: slope of end-systolic pressure–volume relationship (ESPVR), V_{70}: volume intercept of end-systolic pressure–volume relationship at 70 mm Hg, E_a: cardiac afterload, τ: time constant of left ventricular pressure decay, LVEDP: left ventricular end-diastolic pressure, b: diastolic stiffness constant.

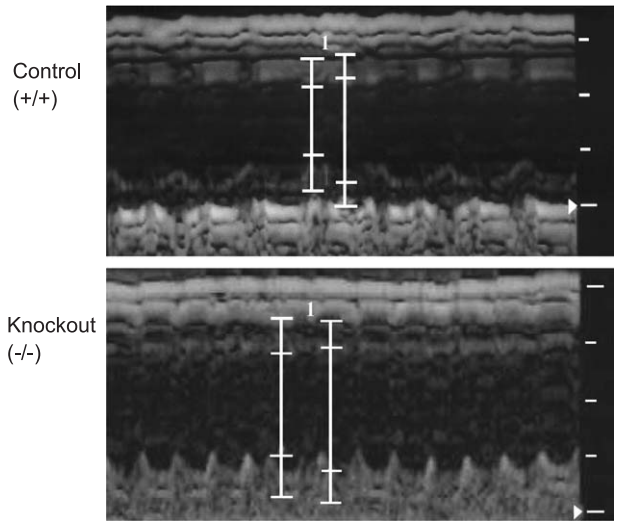

Fig. 1. Representative M-mode analysis of the short cardiac axis in control and knockout mice determined by echocardiography.

animals compared to wild type: Left ventricular end-diastolic diameter was 5.9 ± 0.1 vs. 4.7 ± 0.1 mm ($P < 0.01$), whereas left ventricular wall thickness did not differ between both strains (posterior wall: 0.13 ± 0.01 vs. 0.13 ± 0.01 mm; n.s.; interventricular septum: 0.13 ± 0.02 vs. 0.14 ± 0.01 mm; n.s.) (see Table 1 and Fig. 1). The knockout mice have been characterized by an impaired fractional shortening (left ventricle%fractional shortening [LV%FS]: 34.6 ± 3.2 vs. 19.2 ± 1.2 %). The heart-body weight ratio was not different (5.4 ± 0.1 vs. 5.8 ± 0.5 mg/g).

3.2. Hemodynamics

Systolic blood pressure of angiotensinogen-deficient mice was significantly decreased as described before [14]: 60 ± 3 vs. 90 ± 3 mm Hg, $P < 0.0001$. Heart rate and cardiac output did not differ between the groups (Table 1).

3.3. Ventricular function

Systolic function as determined by ejection fraction, left ventricular pressure max and dP/dt_{max} was significantly depressed in the angiotensinogen-deficient animals (Table 1). The load-independent parameters V_{70} and SdP derived from the systolic pressure–volume relations indicate a significantly decreased myocardial contractility. That there was no significant difference in end-systolic elastance could be due to the fact that the end-systolic pressure–volume relationship is slightly non-linear and the slope of end-systolic elastance is determined at a lower pressure range and thus a relatively steeper part of the end-systolic pressure–volume relationship in the angiotensinogen-deficient animals.

Diastolic function was largely unchanged as indicated by early relaxation parameters (dP/dt_{min} and τ), end-diastolic pressure and end-diastolic stiffness constant b.

3.4. Pressure volume loops

As shown in Fig. 2, the pressure volume loops were shifted to the right. The increased end-systolic and end-diastolic volumes indicate substantial dilation. Cardiac

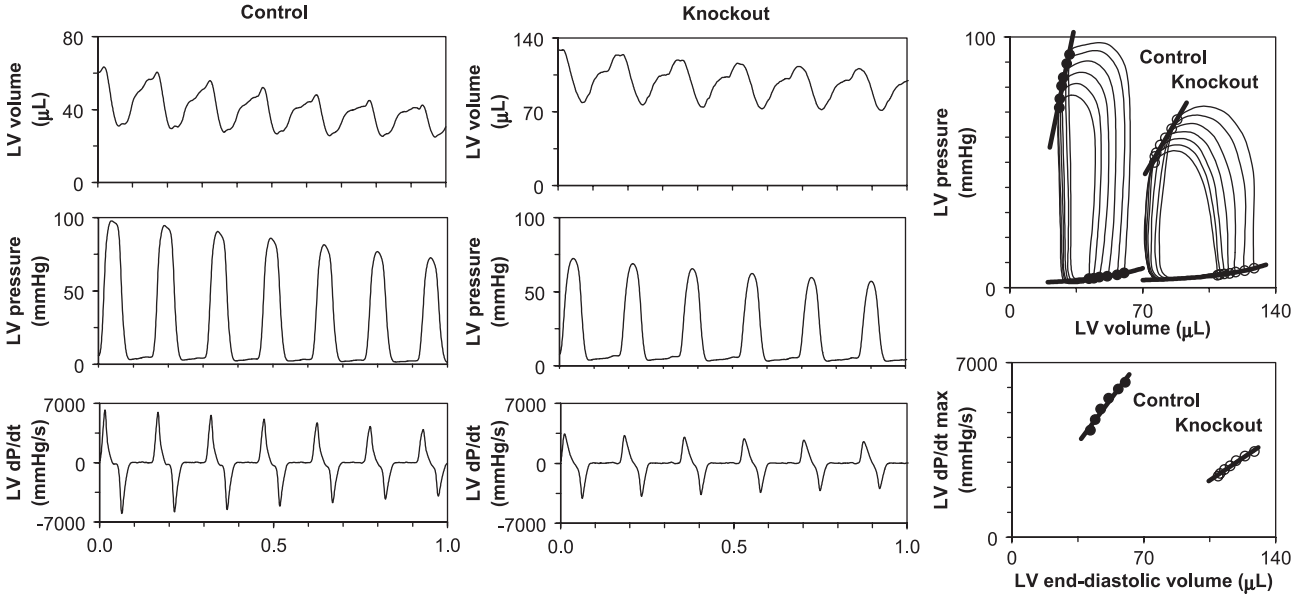

Fig. 2. Left and middle panels: typical left ventricular volume, pressure and dP/dt signals in control and knockout mice during transient preload reduction induced by direct occlusion of the abdominal vena cava inferior. Right panels: Corresponding pressure–volume loops and systolic and diastolic pressure–volume relations, and dP/dt max vs. end-diastolic volume relationships.

output, however, was maintained by strongly reduced afterload.

4. Discussion

In the presence of a primary disturbance in myocardial contractility the heart depends on a number of adaptive mechanisms to maintain cardiac output. In addition to the Frank-Starling mechanism, neuronal and hormonal systems are activated, especially the sympathetic nervous system and the renin-angiotensin system. The latter mechanisms act on both the vasculature and the heart. The capacity of each of these mechanisms to sustain cardiac pump function is finite and, when chronically maintained, becomes maladaptive (Katz, 1990).

Thus, high angiotensin II level is accompanied with cardiac remodeling including hypertrophy, fibrosis and chamber dilation (Sutton and Sharpe, 2000). Surprisingly, we found significant differences in cardiac systolic performance in mice lacking angiotensin II without changes in diastolic compliance. The deficiency of angiotensin peptides leads to LV dilation and in consequence to a shift of the PV loop to the right. However, the cardiac output is maintained presumable due to a reduction in afterload (Devlin et al., 1999). Angiotensinogen-deficient animals are characterized by a marked hypotension, which seems to be more a consequence of a reduced vessel wall tension than a regulating mechanism.

Importantly, as predicted by Nemoto et al. (2002) demonstrating a high correlation between conductance and echocardiography in identifying altered cardiac parameters, the dilation in angiotensinogen-deficient hearts visualized by a shift in pressure volume loops was paralleled by echocardiographic alterations.

Angiotensin II has direct cardiac effects by influencing contractility and cell growth, as also indirect effects by influencing the sympathetic nerve system (Baker et al., 1992). Since angiotensin II regulates cardiac cell growth, its deficiency may lead to structural changes of myocytes and cardiac matrix resulting in a reduction of left ventricular function. However, in the present study, we did not find changes in wall thickness or cardiac weight. In addition, we performed picrosirius red staining and did not find changes in cardiac extracellular matrix content (data not shown).

Most data about the role of cardiac angiotensin II have been obtained by using animal models which have higher concentrations of renin-angiotensin system components in the heart, temporally or continuously (Hein, 1999). Since the angiotensin II-induced phenotypic alterations could be blocked by angiotensin AT_1 receptor antagonist in these studies, the angiotensin AT_1 receptor has been suggested to mediate the main cardiac effects of the peptide. However, the absence of angiotensin AT_1 receptor stimulation in our model leads to dilated cardiomyopathy, that could indicate also a role of the angiotensin AT2 receptor axis. This is also implicated by findings of Ichihara et al. (2001) demonstrating a key role of angiotensin AT_2 in cardiac remodeling. Since also a role of angiotensin AT_2 in embryonic cell division has been demonstrated, we can not exclude that a dysregulation of prenatal cardiogenesis causes the observed phenotype in older animals.

Our data show that pathophysiological changes are not only mediated by over-expression of cardiac or systemic angiotensin but also by the absence of this peptide leading to a dilated cardiomyopathy.

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