

Fibrosis of atria and great vessels in response to angiotensin II or aldosterone infusion

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Abstract

Objective: Myocardial fibrosis, associated with increased expression of angiotensin converting enzyme (ACE) and bradykinin (BK) receptor binding at sites of tissue repair, accompanies chronic elevations in circulating angiotensin II (AngII) and/or aldosterone (ALDO) that simulate chronic cardiac failure. A role for increased ventricular wall stress, associated with arterial hypertension, that can accompany such neurohormonal activation when ventricular function is not compromised, has been held responsible for this structural remodeling. To address this proposition, we monitored morphology of right and left atria and pulmonary artery, where stress is not increased, and compared these structures with hypertensive aorta. **Methods:** Experimental groups included: (1) unoperated and untreated controls; (2) intact rats receiving AngII (9 $\mu\text{g}/\text{h}$) for 2 weeks and which causes arterial hypertension; (3) uninephrectomized control rats on a high sodium diet for 6 weeks; and (4) uninephrectomized rats receiving ALDO (0.75 $\mu\text{g}/\text{h}$) and a high sodium diet for 6 weeks and which results in gradual onset arterial hypertension. Fibrosis was identified by light microscopy in sections stained with collagen specific picrosirius red, while ACE, AngII and BK receptor binding were localized and quantitated by in vitro autoradiography using ^{125}I -351A, $^{125}\text{I}[\text{Sar}^1, \text{Ile}^8]\text{AngII}$, and $^{125}\text{I}[\text{Tyr}^8]\text{BK}$, respectively. AngII receptor subtype was defined by the presence of excess AT_1 (losartan) or AT_2 (PD123177) receptor antagonists, respectively. **Results:** With either AngII or ALDO administration, and compared to controls, we found: (1) microscopic scarring that replaced lost myocytes in both left and right atria; (2) an increase in adventitial collagen of both pulmonary artery and aorta (perivascular fibrosis); (3) markedly increased ACE binding at fibrous tissue sites in both atria and great vessels; (4) unchanged atrial and great vessel AT_1 receptor binding; and (5) significantly increased BK receptor binding at sites of atrial and perivascular fibrosis. **Conclusions:** Thus, the appearance of atrial fibrosis and perivascular fibrosis of aorta and pulmonary artery, together with associated increase in ACE and BK receptor binding, in rats receiving AngII or ALDO suggests these responses are not related to altered ventricular wall stress or arterial hypertension, but rather to these effector hormones of the circulating renin–angiotensin–aldosterone system. Local BK, regulated by ACE found in fibrous tissue and BK receptor binding may play a role in structural remodeling of atria and great vessels in these rat models that simulate chronic cardiac failure. © 1997 Elsevier Science B.V.

Keywords: Fibrosis; Angiotensin receptor; Angiotensin converting enzyme; Autoradiography, in vitro; Bradykinin receptors

1. Introduction

The circulating renin–angiotensin–aldosterone system (RAAS) contributes to cardiovascular system regulation, including the maintenance of salt and intravascular volume homeostasis mediated by its effector hormones, angiotensin (AngII) and aldosterone (ALDO). AngII and ALDO also have functions that contribute to normal

cardiovascular structure during growth and development [1,2] and to adverse structural remodeling in pathologic conditions, such as chronic cardiac failure [1,3]. This laboratory and others have previously reported the appearance of ventricular fibrosis, expressed as microscopic scars and perivascular fibrosis of intramural coronary vessels, in response to unilateral renal artery banding and with chronic administration of either AngII or ALDO in rats with preserved cardiac function that subsequently developed

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arterial hypertension [4–6]. The mechanism responsible for this pathologic structural remodeling of ventricular myocardium has been debated. Hypertension per se has been considered responsible by some [7,8]. Previous studies, however, demonstrated the presence of fibrosis in both the normotensive, nonhypertrophied right and hypertensive, hypertrophied left ventricles in rats with renal ischemia or those receiving either AngII or ALDO [4,9]. Accordingly, it was our hypothesis that such pathologic remodeling is not related to wall stress or hypertension, but instead to these circulating hormones of the RAAS. To address the relationship between such hemodynamic factors versus these hormones and tissue fibrosis, rats received a chronic infusion of either AngII or ALDO. Our first objective was to determine whether fibrous tissue appears in right and left atria and pulmonary artery, none of which is pressure-overloaded in these models, and to compare these structures with pressure-overloaded aorta.

ACE is responsible for AngII generation and in its dual role as a kininase II it contributes to bradykinin (BK) degradation [10]. ACE binding density is markedly increased at sites of tissue repair in the rat heart irrespective of their location and etiologic basis [4,11,12] and independent of circulating AngII [4]. This includes fibrous tissue that appears after myocardial infarction (MI) secondary to coronary artery ligation [11,13], endomyocardial fibrosis following isoproterenol administration [12], myocardial fibrosis with chronic infusion of either AngII or ALDO [4], pericardial fibrosis following pericardiotomy (without MI), and foreign-body fibrosis in response to silk ligature placement in the myocardium [12]. In addition to the heart, high-density ACE binding is found at sites of tissue repair involving other organs, such as the infarcted kidney and foreign-body fibrosis surrounding silk ligature in incised skin [12]. These findings suggest that ACE and its related peptides, AngII or BK, are associated with tissue repair. BK is a chemical mediator of inflammation that appears in injured tissue, including heart [14], and its actions are mediated by BK receptors [15]. In rats receiving either AngII or ALDO, BK receptor binding was found to be significantly increased at sites of fibrosis involving the ventricles [16]. Our second objective, therefore, was to determine the expression of ACE and receptor binding to its related peptides in atria in response to chronic administration of either AngII or ALDO.

2. Methods

2.1. Experimental design

2.1.1. Experiment 1

Eight-week-old male Sprague-Dawley rats were purchased from Harlan Sprague-Dawley (Indianapolis, IN). Four groups ($n = 6$ /group) were studied: (1) unoperated, untreated control rats; (2) subcutaneous AngII (9 $\mu\text{g}/\text{h}$)

given by implanted minipump; (3) uninephrectomy control with 1% dietary NaCl; and (4) uninephrectomized rats who received subcutaneous ALDO (0.75 $\mu\text{g}/\text{h}$) by implanted minipump together with 1% NaCl in drinking water. Previous studies from this laboratory have demonstrated that circulating AngII and ALDO are elevated to levels found in chronic cardiac failure with these models and that each is associated with the subsequent appearance of biventricular fibrosis, arterial hypertension, and left ventricular hypertrophy; right ventricular hypertrophy has not been observed with these models [5,9]. We therefore did not repeat these measurements in the present study. It also was previously demonstrated [4] that ventricular fibrosis was first evident at 2 weeks of AngII administration while it was not seen until week 4 or more with ALDO infusion. In the present study we therefore selected week 2 and 6, respectively, for analysis in these models.

Rats in groups 1 and 2 were killed by overdose of halothane at 2 weeks AngII infusion while rats in groups 3 and 4 were killed at 6 weeks of ALDO administration. Hearts were removed, rinsed in cold saline and frozen in isopentane with dry ice for histopathologic and autoradiographic studies. Serial cryostat coronal sections that included right and left atria, pulmonary artery and aorta were prepared for morphological and autoradiographic analysis.

2.1.2. Experiment 2

Untreated rats ($n = 6$) and rats receiving AngII ($n = 10$) for 2 weeks and rats receiving ALDO for 6 weeks ($n = 6$) were sacrificed, hearts were removed and dissected, left and right atrial, left and right ventricular weights were measured.

2.2. General methods

2.2.1. Collagen morphology

Cryostat coronal (6 μm) sections of each atria were stained with hematoxylin–eosin and collagen-specific staining with picosirius red to identify fibrous tissue. Interstitial collagen volume fraction of atria was determined using videodensitometry and was calculated as the sum of all connective tissue areas of the coronal section, divided by the sum of all connective tissue and muscle areas in all fields of the section as previously reported [9].

2.2.2. Autoradiographic localization of ACE

Radioligand preparation. The radioligand used to label ACE was [^{125}I]351A, a tyrosyl derivative of lisinopril and potent competitive inhibitor of ACE. 351A was iodinated by the chloramine T method and separated from free [^{125}I] by SP Sephadex C25 column chromatography as previously reported [17]. Binding properties of this radioligand have been previously reported [18]. The purity of the radioligand was examined by high-performance liquid chromatography and a single activity peak obtained. The stability of [^{125}I]351A has been previously examined.

ACE binding. Serial 20 μm cryostat sections of hearts were cut in the coronal plane, thaw-mounted onto gelatin-coated slides, and dried in a desiccator at 4°C for 12 h. Sections were incubated in 10 mM sodium phosphate buffer, pH 7.4, containing 150 mM NaCl and bovine serum albumin (2 g/l) with [^{125}I]351A [0.3 $\mu\text{Ci}/\text{ml}$ (~ 300 pM)] for 1 h at 20°C. Nonspecific binding was determined in parallel incubations containing either 1 mM ethylenediamine tetra-acetate (EDTA) [19] or 10 μM unlabeled lisinopril. After incubation, sections were transferred through 4 successive 1 min washes at 0°C, dried under a stream of cold air, placed in X-ray cassettes, and exposed to Kodak NMB-6 film for 3 days.

2.3. Autoradiographic localization of AngII receptors

Cryostat 20 μm coronal sections of heart were preincubated for 15 min in sodium phosphate buffer, then incubated for 1 h in a fresh volume of the same buffer containing 0.2 $\mu\text{Ci}/\text{ml}$ (≈ 90 pM) [^{125}I][Sar¹,Ile⁸]AngII

(Amersham, Arlington Heights, IL), 2 g/l albumin, 0.4 mM bacitracin and 5 mM Na₂EDTA. Nonspecific binding was measured in the presence of 1 μM unlabeled AngII [20]. To characterize AngII receptor subtypes, sections were incubated with either [^{125}I][Sar¹,Ile⁸]AngII in presence of 10 μM of either the AT₁ receptor antagonist, DuP753 (losartan), or the AT₂ receptor antagonist, PD123177. AT₁ receptor binding was determined as that persisting in the presence of an excess of PD123177, while AT₂ receptor binding was defined as that persisting in the presence of an excess of DuP753 [21]. After incubation, sections were washed, dried and exposed to Kodak NMB-6 film for 4 weeks.

2.3.1. Autoradiographic localization of BK receptors

Sections were preincubated for 15 min in 10 mM sodium phosphate buffer (pH 7.4) containing 150 mM NaCl, 5 mM Na₂EDTA, 2 g/l albumin and 0.4 mM bacitracin, then incubated for 1 h in a fresh volume of the same buffer containing 0.2 $\mu\text{Ci}/\text{ml}$ (≈ 90 pM) [^{125}I -

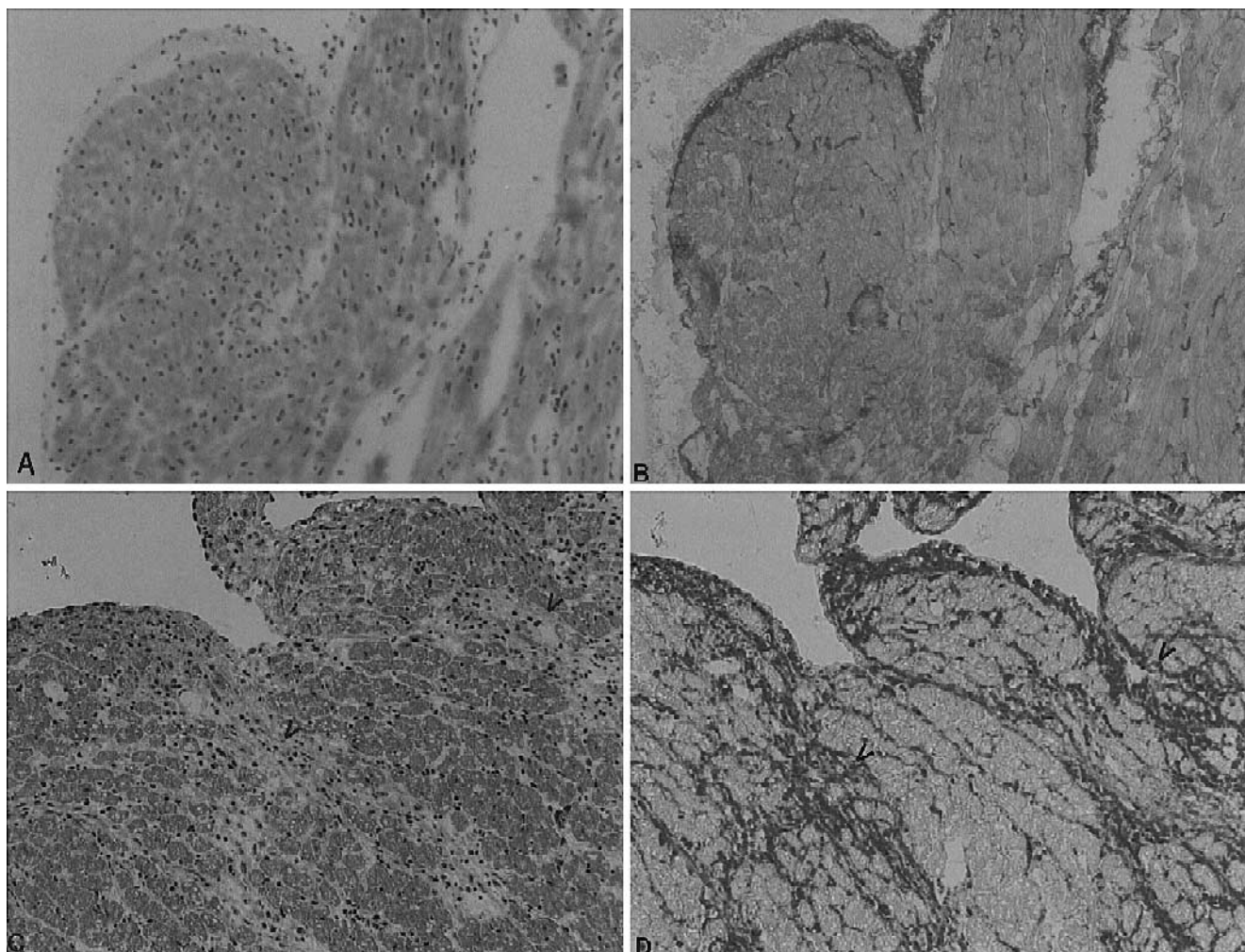


Fig. 1. Atrial fibrosis (arrowheads) in response to AngII. Compared to controls (panels A, H&E and B, PSR). After 2 weeks of AngII treatment, microscopic scars were evident in atria (panel C, H&E). Collagen accumulated at sites of tissue repair (panel D, PSR).

Tyr⁸]BK (Amersham). Specific activity of ¹²⁵I[Tyr⁸]BK is 2200 Ci/mmol. Nonspecific binding was measured in the presence of 1 μM unlabeled BK [22]. After incubation, sections were transferred through 4 successive 1 min washes at 0°C, dried under a stream of cold air, placed in X-ray cassettes and exposed to Kodak NMB-6 film for 4 weeks.

2.3.2. Analysis of ACE, AngII and BK receptor binding densities

¹²⁵I radioactivity standards (Amersham) were exposed to the same film in parallel tissue sections. Quantitation of binding density was performed using a computer-image-analyzed system (Quantimet 520, Cambridge Lab. Inc., MA). Radioactivity standards were corrected for decay and fitted to calibration curves by the computer to convert optical density values of each pixel into ¹²⁵I radioactivity expressed as dpm/mm².

2.3.3. ACE, AngII or BK receptor binding regions in the heart

After exposure, sections were stained with hematoxylin and eosin, overlapped to radiographic film, and examined

by light microscopy to determine the distribution of ACE, AngII or BK receptors.

2.4. Statistical analysis

Statistical analyses of autoradiographic findings, collagen volume fraction and heart weight were performed using Student's *t*-test. Values are expressed as means ± s.e.m. with *P* < 0.01 considered significant.

3. Results

3.1. Morphologic findings

Compared with unoperated, untreated controls, where no evidence of atrial fibrosis was observed (Fig. 1, panels A and B), microscopic scars were found scattered throughout both atria in rats receiving AngII for 2 weeks (Fig. 1, panels C and D). In rats with uninephrectomy and high salt diet alone for 6 weeks, no histological evidence of fibrosis was observed in either atria and served as control to uninephrectomized rats receiving ALDO and a high salt

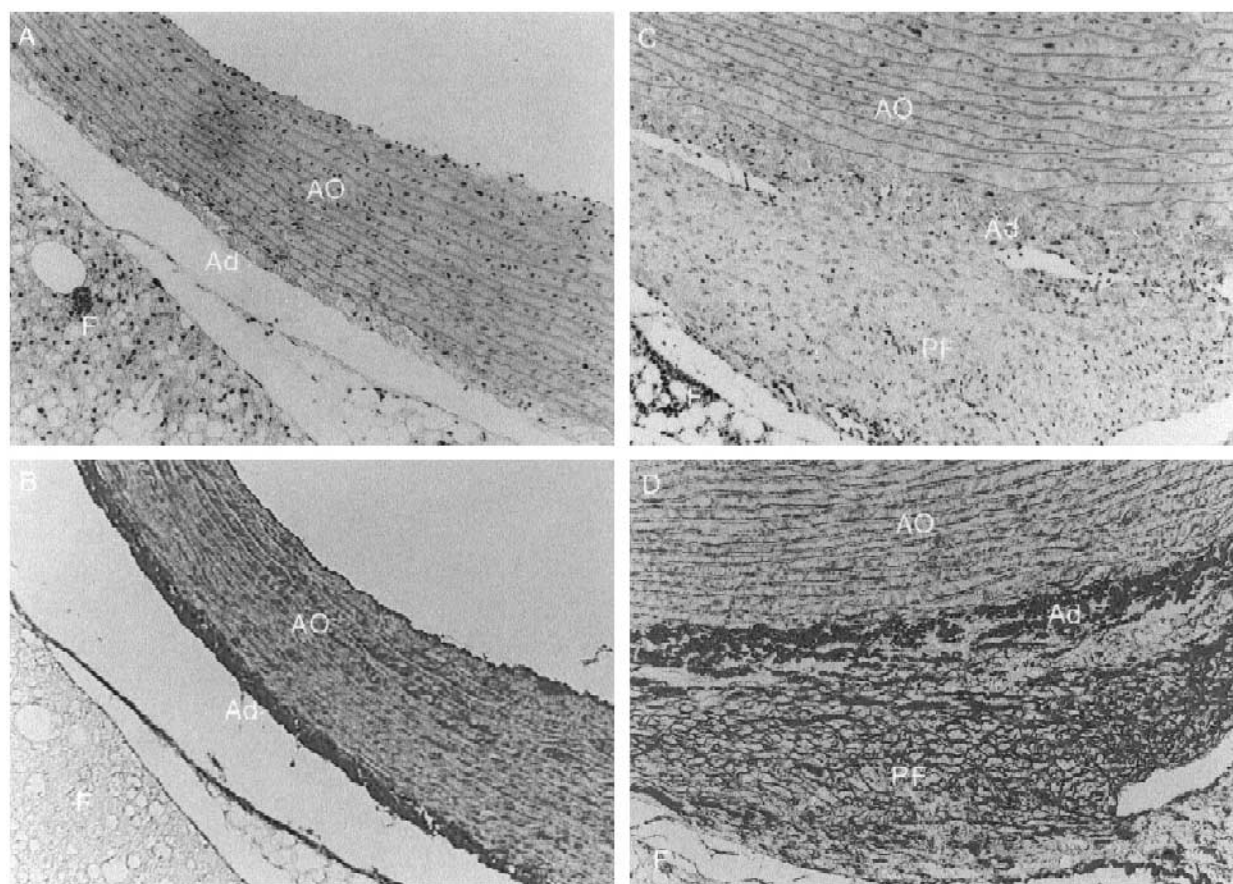


Fig. 2. Perivascular fibrosis of aorta in rats receiving chronic administration of AngII. Panels A (H&E) and B (PSR) show the aorta (AO) with small amount of collagen in adventitia (Ad) in a control rat. Panels C and D show the aorta with perivascular fibrosis (PF) in a rat receiving AngII infusion. F = fat tissue surrounding aorta.

diet. In this latter group, microscopic scars were diffusely distributed throughout both atria. Quantitative data show that interstitial collagen volume fraction of atria was significantly ($P < 0.01$) increased in rats receiving AngII ($4.87 \pm 0.21\%$) or ALDO ($4.99 \pm 0.28\%$) compared to controls ($2.94 \pm 0.31\%$).

Compared with their respective controls, where a small amount of collagen was observed in adventitia, perivascular fibrosis (i.e., increase in adventitial collagen) was seen in both aorta and pulmonary artery in rats receiving AngII for 2 weeks (Fig. 2) and in rats receiving ALDO for 6 weeks.

3.2. Autoradiographic ACE binding

ACE binding was observed in right and left atria of control groups 1 and 3. However, quantitative binding was of low to moderate density (Fig. 3, panel A; Table 1). In rats receiving AngII, ACE binding was significantly ($P < 0.01$) increased in both right and left atria (Fig. 3, panel B; Fig. 4, panel B; Table 1). Such high ACE binding was coincident with sites of microscopic scars seen in serial

coronal sections (Fig. 4, panel A). In rats receiving ALDO for 6 weeks, ACE binding was also markedly increased in each atrium (Fig. 3, panel C; Table 1) and, once again, high-density ACE binding was seen at sites of microscopic scars scattered throughout both right and left atria (Fig. 3, panel C).

High-density ACE binding appeared in endothelium and adventitia of aorta of control rats (Fig. 3, panel A). In rats receiving AngII (Fig. 3, panel B; Fig. 4, panels C and D) or ALDO (Fig. 3, panel C), high ACE binding was seen at the site of perivascular fibrosis of aorta. Moderate-density ACE binding is normally present in pulmonary artery (Fig. 3, panel A). In rats receiving AngII or ALDO for 2 and 6 weeks, respectively, ACE binding was markedly increased in perivascular fibrosis of pulmonary artery adventitia (Fig. 3, panels B and C; Table 1).

3.3. Autoradiographic AngII receptor binding

AngII receptor binding was present in both right and left atria of normal control rats (Fig. 3, panel D). Quantita-

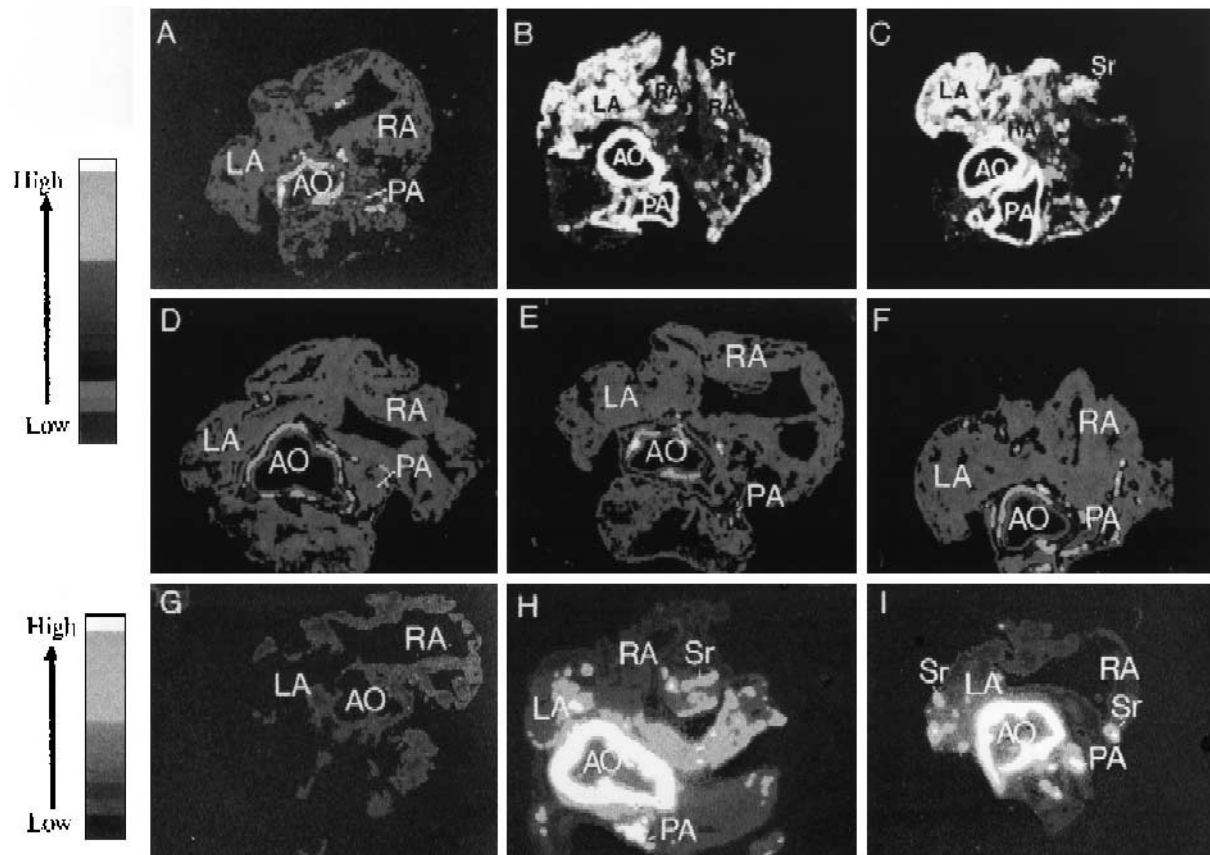


Fig. 3. Autoradiographic localization and binding density of ACE, AngII and BK receptors in rat atria in response to either AngII or ALDO (computerized pictures). In control rats, ACE binding is normally low in atria (LA, RA), moderate in pulmonary artery (PA) and high in aorta (AO). In rats receiving AngII (panel B) or ALDO (panel C), ACE binding was markedly increased in microscopic scars (Sr) that appear in atria, as well as perivascular fibrosis of PA and AO. AngII receptor binding was normally low in control atria, AO, and PA (panel D). After AngII (panel E) or ALDO (panel F) treatment, AngII receptor binding was not significantly different from control. Low BK receptor binding is present in normal atria, PA and AO (panel G). BK receptor binding was markedly increased at atrial sites of microscopic scarring and perivascular fibrosis of AO and PA in the rats receiving AngII (panel H) or ALDO (panel I).

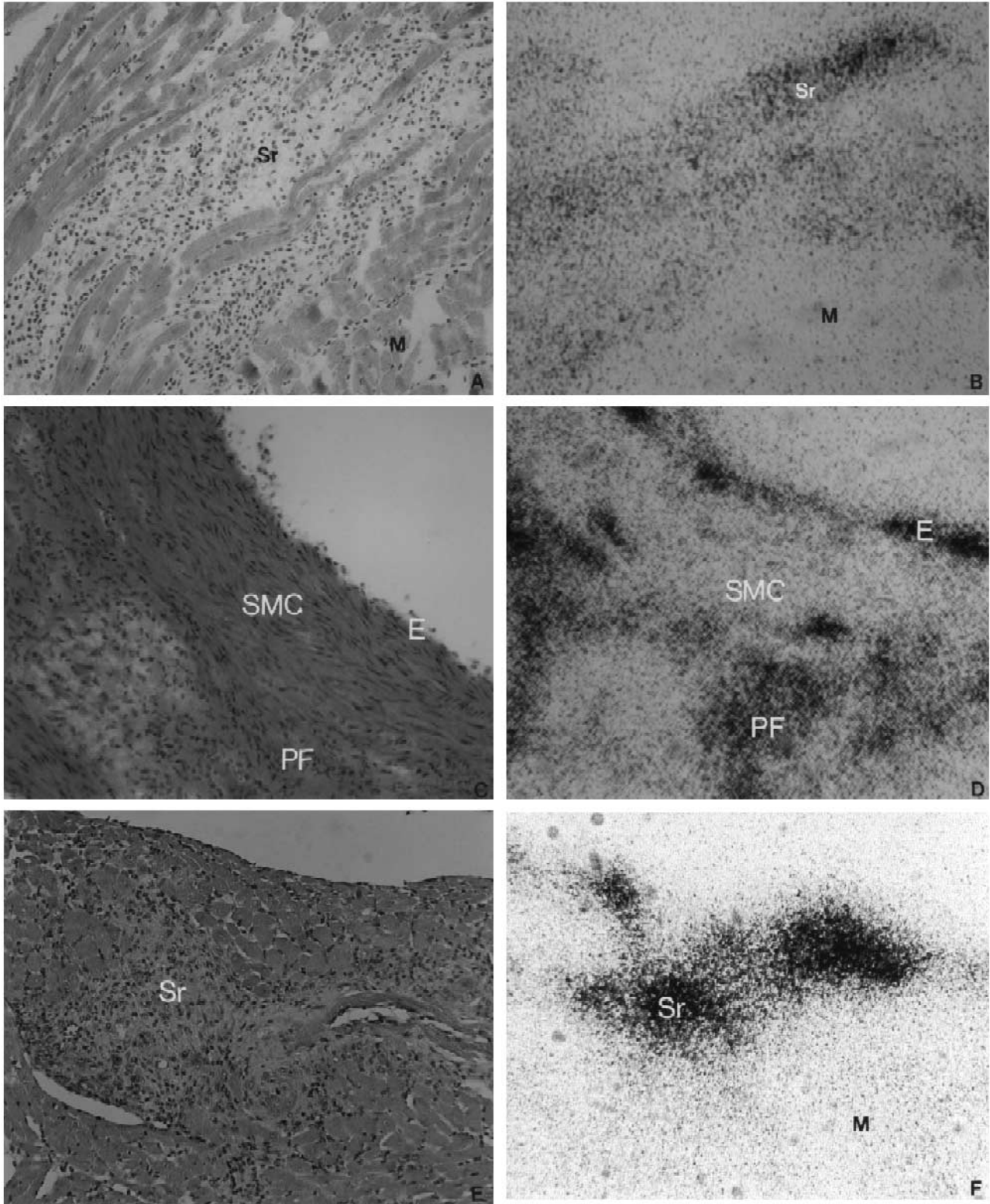


Fig. 4. ACE binding in microscopic scars and perivascular fibrosis of aorta and BK receptor binding in microscopic scars in rats receiving AngII (microscopic pictures). Panel A (H&E) shows microscopic scar (Sr) in the right atrium. In the adjacent section, ACE binding (panel B, silver grains) was markedly increased compared to myocardium of atria (M). Panel C shows an aorta with perivascular fibrosis (PF). In the serial section, ACE binding was high in endothelium (E) and the site of perivascular fibrosis and low in smooth muscle cells (SMC). At the site of microscopic scar in the right atrium (panel E), BK receptor binding was markedly increased compared to myocardium of atria.

Table 1

ACE, AngII and BK receptor binding densities in atria, aorta, pulmonary artery following chronic administration of either angiotensin II (AngII) or aldosterone (ALDO)

Groups	Binding densities (dpm/mm ²)		
	ACE	AngIIR	BKR
<i>Control</i>			
LA	256.4 ± 13.8	11.2 ± 2.4	12.5 ± 1.9
RA	302.9 ± 20.4	10.5 ± 1.9	9.8 ± 1.0
APA	453.7 ± 34.2	28.4 ± 1.2	12.3 ± 1.9
AAO	714.8 ± 51.4	32.1 ± 5.3	13.2 ± 2.1
<i>AngII</i>			
LA	358.2 ± 41.2 ^a	9.4 ± 1.8	20.9 ± 2.7 ^a
RA	389.7 ± 46.3 ^a	9.8 ± 2.4	21.8 ± 2.0 ^a
Sr	893.2 ± 79.1	11.3 ± 1.9	39.4 ± 4.9
PFFPA	732.8 ± 89.4 ^a	30.4 ± 2.7	38.9 ± 2.7 ^a
PFAO	936.4 ± 75.1 ^a	34.1 ± 4.2	35.43.6 ^a
<i>ALDO</i>			
LA	321.3 ± 25.4 ^a	11.9 ± 2.0	23.1 ± 3.4 ^a
RA	372.2 ± 23.6 ^a	13.2 ± 2.7	18.9 ± 1.4 ^a
Sr	751.8 ± 32.7	9.2 ± 1.9	43.5 ± 6.1
PFFPA	719.4 ± 43.2 ^a	29.4 ± 4.3	34.1 ± 5.2 ^a
PFAO	978.1 ± 69.4 ^a	30.6 ± 3.7	30.2 ± 6.0 ^a

LA = left atrium; RA = right atrium; APA = adventitia of pulmonary artery; AAO = adventitia of aorta; PFFPA = perivascular fibrosis of pulmonary artery; PFAO = perivascular fibrosis of aorta; Sr = microscopic scars.

^a $P < 0.01$.

tive binding density, however, was low (see Table 1). In rats receiving either AngII (Fig. 3, panel E) or ALDO (panel F), AngII receptor binding in atria was not significantly different compared to controls. AngII receptor binding was displaced by the AT₁ receptor antagonist, losartan, but not the AT₂ receptor antagonist, PD123177, indicating that the predominant subtype of AngII receptors in rat atria, aorta and pulmonary artery is AT₁ (not shown). Similar autoradiographic displacement binding data have previously been reported for right and left ventricles in these models and normal controls [4].

3.4. Autoradiographic BK receptor binding

Very low BK receptor binding was observed in both atria of normal untreated control rats (Fig. 3, panel G). In rats receiving either AngII (Fig. 3, panel H) or ALDO (Fig. 3, panel I), BK receptor binding was significantly ($P < 0.01$) increased at sites of microscopic scars (Fig. 4, panels E and F) in both right and left atria compared to control (see Table 1). BK receptor binding was markedly increased at sites of microscopic scarring found in both atria (Fig. 3, panels H and I) in each model.

3.5. Heart weight

Compared to controls, left ventricular weight was significantly increased in rats receiving AngII or ALDO,

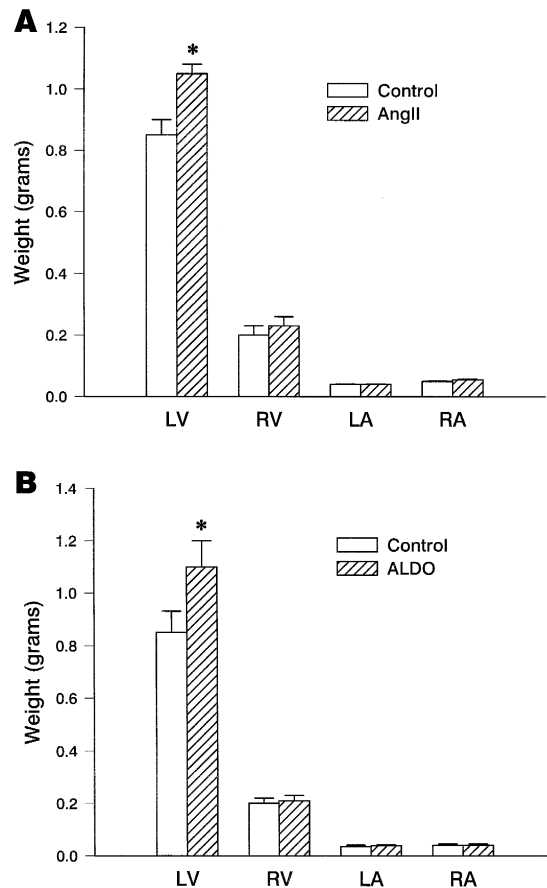


Fig. 5. Heart weight in rats receiving AngII (panel A) and ALDO (panel B). In each model, weight of left ventricle was significantly increased ($P < 0.01$) compared to controls. Weight of right ventricle and atrium was not changed.

while right ventricular, left and right atrial weights remained unchanged (Fig. 5).

4. Discussion

Chronic, inappropriate (relative to sodium intake and intravascular volume) elevations in circulating AngII and/or ALDO, such as can occur in chronic cardiac failure or various forms of arterial hypertension, have each been associated with cardiac myocyte necrosis followed by fibrillar collagen accumulation in the form of microscopic scarring [23,24]. This reparative fibrosis of both right and left ventricles has been observed with endogenous activation of the renin–angiotensin–aldosterone system, created by unilateral renal artery ischemia [25], or exogenous AngII or ALDO administration [5]. Chronic administration of AngII in previously healthy rats or ALDO in uninephrectomized rats on a high salt diet each leads to ventricular fibrosis that includes microscopic scarring [4,5]. The time course for the appearance of this pathological remodeling, however, differs in these two animal models as noted earlier and, therefore, different mechanisms may be operative.

Our first objective in the present study was to determine whether fibrosis is present in right and left atria and pulmonary artery, where wall stress is not elevated, and aorta where wall stress is increased in both AngII or ALDO models. Elevated systolic wall stress has been considered relevant to the appearance of fibrosis involving the left ventricle [6,7]; however, this is controversial [4,6,7,9]. Wall stress is elevated in the hypertensive left ventricle and could be increased in the right ventricle in response to chronic pulmonary venous hypertension; however, right ventricular hypertrophy that would accompany such enhanced loading has not been observed in these models [9,26,27]. Fibrosis appears not only in the hypertensive, hypertrophied left ventricle, but also the normotensive, nonhypertrophied right ventricle [4,9]. This is in keeping with increased collagen mRNA expression in each ventricle while increased expression of mRNA for atrial natriuretic peptide, a marker of ventricular hypertrophy, is seen only in the left ventricle [27]. Fibrosis in each of these models is therefore not related to arterial hypertension, elevated systolic wall stress, or cardiac myocyte hypertrophy [26–28]. In the absence of right ventricular pressure overload and hypertrophy in these models, right atrial overload and hypertrophy are not expected. Our data of heart weight further indicate the absence of hypertrophy in right ventricle and atria. Perivascular fibrosis of adventitia, defined here as an expansion of adventitial collagen, appeared in the pulmonary artery, without increased wall stress, and in the aorta with increased wall stress. Our findings further support the proposition that the appearance of ventricular and atrial fibrosis and perivascular fibrosis of great vessels in these models is not related to altered wall stress, arterial hypertension or ventricular hypertrophy.

Bilateral total or subtotal (medulla) adrenalectomy was found to prevent myocardial scarring and presumably myocyte loss in the AngII infusion model [29]. This was not the case for co-administration of the ALDO receptor antagonist, spironolactone, suggesting that AngII-induced catecholamine release from the adrenal medulla is a primary cause of myocyte necrosis with subsequent scarring in this model. Myocyte loss with scarring accompanies chronic mineralocorticoid treatment and has been related to potassium loss and can be prevented by spironolactone [30], a potassium-sparing diuretic, amiloride [31], or dietary potassium chloride supplementation [32]. An explanation for the expansion of great vessel adventitial collagen with either AngII or ALDO administration is presently uncertain. Previous studies with this AngII infusion model have demonstrated increased vascular permeability [29,33,34] mediated by the release of nitric oxide and which could be prevented by L-NAME [35,36]. Whether such hyperpermeability appeared in these great vessels is unclear, but would seem unlikely. A direct effect of AngII or ALDO on fibroblasts located within the adventitia is a potential explanation and is suggested by studies in cultured adult

rat cardiac fibroblasts, where collagen synthesis is increased by either hormone [37,38].

The second objective of this study was to determine the expression of ACE and receptor binding to its related peptides, AngII and BK, in atria, pulmonary artery and aorta in response to chronic administration of either AngII or ALDO. High-density ACE binding was observed at sites of microscopic scars scattered throughout both right and left atria in each model. Increased BK receptor, but low or normal AngII receptor binding was co-localized with ACE at these sites of remodeling. Similar results has been observed in each ventricle in these models [4]. The coincidence of increased ACE binding and BK receptor binding at sites of fibrosis suggests that ACE may be responsible for metabolizing BK which, in turn, plays a role in tissue repair. We have previously demonstrated that an ACE inhibitor, lisinopril, significantly attenuated collagen accumulation at expected sites of fibrosis associated with AngII administration [39]. The attenuation in collagen formation by lisinopril is not likely due to reduced circulating AngII levels, since circulating AngII is chronically increased in association with continuous exogenous AngII administration, or to reduced arterial pressure since the dose of lisinopril selected did not prevent arterial hypertension that accompanied AngII infusion. Therefore, BK may be responsible for either increased collagen degradation or reduced fibroblast collagen synthesis seen in association with ACE inhibitor treatment in this animal model. In rats receiving AngII, a type 2 BK receptor antagonist, Hoe140, significantly attenuated collagen accumulation in the right and left ventricles [36]. This apparent paradox could be explained by interference with BK receptor binding that reduces inflammatory cell responses which contribute to cell–cell signaling and attenuated subsequent fibroblast fibrogenesis. These cellular events remain to be examined in more detail.

ACE exists predominantly as a membrane-bound ectoenzyme located on endothelial cells [40], epithelial cells [41], macrophages [42] and fibroblast-like cells [43,44]. Increased ACE binding in the injured heart, such as follows myocardial infarction, is independent of circulating renin, soluble ACE and ALDO [45,46]. In the present study, circulating AngII and ALDO were chronically elevated in rats receiving AngII [5] while circulating ALDO, but not AngII, was increased in uninephrectomized rats receiving ALDO [9]. In both models, however, ACE binding density was markedly increased at sites of atrial scarring and perivascular fibrosis of great vessels. Markedly increased ACE binding has also been found at sites of tissue repair involving other organs [12]. ACE expression by cells involved in tissue repair is therefore not related to hypertension or downregulated by circulating effector hormones of the RAAS. Elevated ACE binding at sites of tissue repair is due to the influx of ACE-expressing cells, such as macrophages and phenotypically transformed fibroblast-like cells, termed ‘myofibroblasts’ because they

express α -smooth muscle actin [47]. In experimental myocardial infarction, produced by ligating the left coronary artery, myocardial hypertrophy is evident in the noninfarcted left ventricle. Passier [48] demonstrated that renin and ACE gene expressions were significantly increased at the site of infarction, but not in hypertrophied, noninfarcted myocardium and therefore intracardiac expression of ACE does not play a role in the development of cardiac hypertrophy, but suggests a role for this ectoenzyme in wound healing [48].

In the infarcted rat heart, AT₁ receptor binding was markedly increased at sites of fibrosis, where cells expressing AT₁ receptors were primarily myofibroblasts [43]. However, AT₁ receptor binding was not significantly increased at sites of fibrosis in either AngII or ALDO model. AngII receptors are cell membrane-bound receptors. In both AngII or ALDO models, AT₁ receptor-containing myofibroblasts were much fewer compared to infarcted heart, which may be a reason for unchanged AT₁ receptor binding at sites of fibrosis. In the AngII model, elevated circulating AngII also downregulated AngII receptors, which led to low AngII receptor binding at sites of fibrosis.

In summary, chronic administration of either AngII to normal rats for 2 weeks or ALDO to uninephrectomized rats plus high salt diet for 6 weeks led to myocyte loss with microscopic scars that appeared in both right and left atria and pulmonary artery, neither of which is pressure-overloaded. The appearance of atrial fibrosis and perivascular fibrosis of great vessels suggests that remodeling of the myocardium by fibrous tissue is not related to ventricular wall stress. Atrial fibrosis may explain the appearance of atria arrhythmias observed in mineralocorticoid-treated rats [49] and could further account for the common occurrence of such dysrhythmias in patients with chronic cardiac failure. ACE and BK receptor binding were significantly increased and co-localized to sites of fibrosis, suggesting that local BK may play a role in tissue repair of the rat heart and related structures in these animal models.

Acknowledgements

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References

- [1] Keeley FW, Elmoselhi A, Leenen FHH. Enalapril suppresses normal accumulation of elastin and collagen in cardiovascular tissues of growing rats. *Am J Physiol* 1992;262:H1013–H1021.
- [2] Baker KM, Booz GW, Dostal DE. Cardiac actions of angiotensin II: role of an intracardiac renin–angiotensin system. *Annu Rev Physiol* 1992;54:227–241.
- [3] Weber KT, Brilla CG. Pathological hypertrophy and cardiac interstitium: fibrosis and renin–angiotensin–aldosterone system. *Circulation* 1991;83:1849–1865.
- [4] Sun Y, Ratajska A, Zhou G, Weber KT. Angiotensin converting enzyme and myocardial fibrosis in the rat receiving angiotensin II or aldosterone. *J Lab Clin Med* 1993;122:395–403.
- [5] Tan LB, Jalil JE, Pick R, Janicki JS, Weber KT. Cardiac myocyte necrosis induced by angiotensin II. *Circ Res* 1991;69:1185–1195.
- [6] Schunkert H, Dzau VJ, Tang SS, Hirsch AT, Apstein CS, Lorell BH. Increased rat cardiac angiotensin converting enzyme activity and mRNA expression in pressure overload left ventricular hypertrophy. Effects on coronary resistance, contractility, and relaxation. *J Clin Invest* 1990;86:1913–1920.
- [7] Sawamura I, Hazama F, Kinoshita M. Histological and histometrical study of myocardial fibrosis in spontaneously hypertensive rats of the stroke-prone strain. *Jpn Circ J* 1990;54:1274–1282.
- [8] Sugihara N, Genda A, Shimizu M, et al. Quantitation of myocardial fibrosis and its relation to function in essential hypertension and hypertrophic cardiomyopathy. *Clin Cardiol* 1988;11:771–778.
- [9] Brilla CG, Pick R, Tan LB, Janicki JS, Weber KT. Remodeling of the rat right and left ventricle in experimental hypertension. *Circ Res* 1990;67:1355–1364.
- [10] Ehlers MRW, Riordan JF. Angiotensin-converting enzyme: new concepts concerning its biological role. *Biochemistry* 1989;28:5311–5318.
- [11] Sun Y, Cleutjens JPM, Diaz-Arias AA, Weber KT. Cardiac angiotensin converting enzyme and myocardial fibrosis in the rat. *Cardiovasc Res* 1994;28:1423–1432.
- [12] Sun Y, Weber KT. Angiotensin-converting enzyme and wound healing in diverse tissues of the rat. *J Lab Clin Med* 1996;127:94–101.
- [13] Fabris B, Jackson B, Kohzuki M, Perich R, Johnston CI. Increased cardiac angiotensin-converting enzyme in rats with chronic heart failure. *Clin Exp Pharmacol Physiol* 1990;17:309–314.
- [14] Regoli D, Barabé J. Pharmacology of bradykinin and related kinins. *Pharmacol Rev* 1980;32:1–46.
- [15] Burch RM, Kyle DJ. Recent developments in the understanding of bradykinin receptors. *Life Sci* 1992;50:829–838.
- [16] Sun Y, Ratajska A, Weber KT. Bradykinin receptor and tissue ACE binding in myocardial fibrosis: response to chronic angiotensin II or aldosterone administration in rats. *J Mol Cell Cardiol* 1995;27:813–822.
- [17] Yamada H, Fabris B, Allen AM, Jackson B, Johnston CI, Mendelsohn FAO. Localization of angiotensin converting enzyme in rat heart. *Circ Res* 1991;68:141–149.
- [18] Mendelsohn FAO. Localization of angiotensin converting enzyme in rat forebrain and other tissues by in vitro autoradiography using ¹²⁵I-labelled MK351A. *Clin Exp Pharmacol Physiol* 1984;11:431–435.
- [19] Chai SY, Mendelsohn FAO, Paxinos G. Angiotensin converting enzyme in rat brain visualized by quantitative in vitro autoradiography. *Neuroscience* 1987;20:615–627.
- [20] Sun Y, Weber KT. Angiotensin II receptor binding following myocardial infarction in the rat. *Cardiovasc Res* 1994;28:1623–1628.
- [21] Song K, Zhuo J, Allen AM, Paxinos G, Mendelsohn FAO. Angiotensin II receptor subtypes in rat brain and peripheral tissues. *Cardiology* 1991;79(Suppl 1):45–54.
- [22] Baraniuk JN, Lundgren JD, Mizoguchi H, et al. Bradykinin and respiratory mucous membranes. Analysis of bradykinin binding site distribution and secretory responses in vitro and in vivo. *Am Rev Respir Dis* 1990;141:706–714.
- [23] Gavras H, Kremer D, Brown JJ, et al. Angiotensin- and norepinephrine-induced myocardial lesions: experimental and clinical studies in rabbits and man. *Am Heart J* 1975;89:321–332.
- [24] Giacomelli F, Anversa P, Wiener J. Effect of angiotensin-induced hypertension on rat coronary arteries and myocardium. *Am J Pathol* 1976;84:111–138.
- [25] Kremer D, Lindop G, Brown WCB, Morton JJ, Robertson JIS.

- Angiotensin-induced myocardial necrosis and renal failure in the rabbit: distribution of lesions and severity in relation to plasma angiotensin II concentration and arterial pressure. *Cardiovasc Res* 1981;15:43–46.
- [26] Young M, Fullerton M, Dilley R, Funder J. Mineralocorticoids, hypertension, and cardiac fibrosis. *J Clin Invest* 1994;93:2578–2583.
- [27] Robert V, Van Thiem N, Cheav SL, Mouas C, Swynghedauw B, Delcayre C. Increased cardiac types I and III collagen mRNAs in aldosterone-salt hypertension. *Hypertension* 1994;24:30–36.
- [28] Young M, Head G, Funder J. Determinants of cardiac fibrosis in experimental hypermineralocorticoid states. *Am J Physiol* 1995;269:E657–E662.
- [29] Ratajska A, Campbell SE, Cleutjens JPM, Weber KT. Angiotensin II and structural remodeling of coronary vessels in rats. *J Lab Clin Med* 1994;124:408–415.
- [30] Brilla CG, Weber KT. Reactive and reparative myocardial fibrosis in arterial hypertension in the rat. *Cardiovasc Res* 1992;26:671–677.
- [31] Campbell SE, Janicki JS, Matsubara BB, Weber KT. Myocardial fibrosis in the rat with mineralocorticoid excess: prevention of scarring by amiloride. *Am J Hypertens* 1993;6:487–495.
- [32] Darrow DC, Miller HC. The production of cardiac lesions by repeated injections of desoxycorticosterone acetate. *J Clin Invest* 1942;21:601–611.
- [33] Reddy HK, Campbell SE, Janicki JS, Zhou G, Weber KT. Coronary microvascular fluid flux and permeability: influence of angiotensin II, aldosterone and acute arterial hypertension. *J Lab Clin Med* 1993;121:510–521.
- [34] Reddy HK, Sigusch H, Zhou G, Tyagi SC, Janicki JS, Weber KT. Coronary vascular hyperpermeability and angiotensin II. *J Lab Clin Med* 1995;126:307–315.
- [35] Sigusch HH, Ou R, Katwa LC, et al. Angiotensin-II-induced increase in transcortin protein clearance: role of hypertension vs. nitric oxide or cyclo-oxygenase products. *Cardiovasc Res* 1995;30:291–298.
- [36] Sigusch HH, Campbell SE, Weber KT. Angiotensin II-induced myocardial fibrosis in rats: role of nitric oxide, prostaglandins and bradykinin. *Cardiovasc Res* 1996;31:546–554.
- [37] Brilla CG, Zhou G, Matsubara L, Weber KT. Collagen metabolism in cultured adult rat cardiac fibroblasts: response to angiotensin II and aldosterone. *J Mol Cell Cardiol* 1994;26:809–820.
- [38] Zhou G, Kandala JC, Tyagi SC, Katwa LC, Weber KT. Effects of angiotensin II and aldosterone on collagen gene expression and protein turnover in cardiac fibroblasts. *Mol Cell Biochem* 1996;154:171–178.
- [39] Sun Y, Ratajska A, Weber KT. Inhibition of angiotensin-converting enzyme and attenuation of myocardial fibrosis by lisinopril in rats receiving angiotensin II. *J Lab Clin Med* 1995;126:95–101.
- [40] Ryan JW, Ryan US, Schultz DR, Whitaker C, Chung A. Subcellular localization of pulmonary angiotensin-converting enzyme (kininase II). *Biochem J* 1975;146:497–499.
- [41] Hall ER, Kato J, Erdos EG, Robinson CJ, Oshima G. Angiotensin I-converting enzyme in the nephron. *Life Sci* 1976;18:1299–1303.
- [42] Weinstock JV, Boros DL, Gee JB. Enhanced granuloma angiotensin I converting enzyme activity associated with modulation in murine schistosomiasis. *Gastroenterology* 1981;81:48–53.
- [43] Sun Y, Weber KT. Angiotensin converting enzyme and myofibroblasts during tissue repair in the rat heart. *J Mol Cell Cardiol* 1996;28:851–858.
- [44] Katwa LC, Ratajska A, Cleutjens JPM, et al. Angiotensin converting enzyme and kininase-II-like activities in cultured valvular interstitial cells of the rat heart. *Cardiovasc Res* 1995;29:57–64.
- [45] Hodsman GP, Kohzuki M, Howes LG, Sumithran E, Tsunoda K, Johnston CI. Neurohumoral responses to chronic myocardial infarction in rats. *Circulation* 1988;78:376–381.
- [46] Hirsch AT, Talsness CE, Schunkert H, Paul M, Dzau VJ. Tissue-specific activation of cardiac angiotensin converting enzyme in experimental heart failure. *Circ Res* 1991;69:475–482.
- [47] Johnston CI. Tissue angiotensin converting enzyme in cardiac and vascular hypertrophy, repair, and remodeling. *Hypertension* 1994;23:258–268.
- [48] Passier PCJJ. The intracardiac renin–angiotensin system: new insights in its role in cardiac hypertrophy and wound healing [Dissertation]. Rijksuniversiteit Limburg Maastricht, 1995: 156 pp.
- [49] Carré F, Lessard Y, Coumel P, et al. Spontaneous arrhythmias in various models of cardiac hypertrophy and senescence of rats. A Holter monitoring study. *Cardiovasc Res* 1992;26:698–705.