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Enhanced cardiac angiotensinogen gene expression and angiotensin converting enzyme activity in tachypacing-induced heart failure in rats

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Summary: The aim of the study was to analyze changes in myocardial angiotensinogen gene expression and myocardial angiotensin converting enzyme activity in slowly progressing low-output failure. In adult, male Wistar rats, acute ventricular tachypacing by 610 to 620 impulses per minute lowered enddiastolic external diameter of the left ventricle by 2.6% ($p < 0.01$), but did not lower cardiac output or abolish coronary reserve, since left-ventricular subendocardial blood flow of paced rats increased under dipyridamole (2 mg/kg i.v.) by 56% ($p < 0.01$). Systemic neuroendocrine activation and ventricular dilation without enlargement of ventricular mass developed subsequent to chronic tachypacing, but left-ventricular diameter during pacing never exceeded the value of sham rats on sinus rhythm. After 2 weeks, cardiac output was lowered by 14% ($p < 0.001$), cardiopulmonary blood volume was elevated by 30% ($p < 0.001$), and angiotensinogen mRNA and angiotensin converting enzyme activity in ventricular myocardium were doubled. We conclude that conditions for an enhanced intracardiac angiotensin II-formation developed in tachypacing-induced heart failure, but that enhanced systolic wall stress or myocardial ischemia are not required for this activation of the local cardiac renin-angiotensin system.

Key words: Congestive heart failure; cardiac renin-angiotensin system; neuroendocrine activity; coronary reserve; ventricular wall stress; model of failure development

Introduction

Hemodynamic overload (i.e., enhanced systolic myocardial wall stress) induces a trophic response of the heart and a reprogramming of cardiocyte gene expression (23, 29, 33, 41). The functional consequences of this load-induced reprogramming probably contribute to the progressive pump dysfunction in overload cardiomyopathy (23). However, changes in cardiocyte phenotype probably are not only induced by changes in myocardial wall stress.

Experimental observations in vivo indicate that cardiac trophic responses to hemodynamic overload can be modulated by inhibitors of angiotensin converting enzyme (ACE), partially by a load-independent mechanism (27). Such load-independent modulations of cardiac trophic responses appear important for the cardioprotective effects of ACE-inhibition in developing heart failure. Recently, it was speculated that changes in local myocardial angiotensin formation are involved in load-independent modulation of heart failure progression (18). The heart expresses all components necessary for local angiotensin formation (25), angiotensin exerts trophic actions on cardiocytes in cell culture (1, 20, 22, 34) and augments sympathetic transmission in the heart (48, 50), thereby modulating another trophic influence on the myocardium (43, 49).

The testing of load-independent trophic influences on the heart for development and progression of cardiac failure should be facilitated by experimental models of heart failure, which do not involve myocardial overdistension or ischemia as noxious interventions. The low-output failure induced by chronic ventricular tachypacing (3, 37) could be such a model. While the mechanisms causing myocardial impairment in this model are not clear, it has been frequently applied in dogs to analyze neuroendocrine regulation in heart failure (9, 19, 30, 32, 38, 46, 47).

We tested in rats whether low-output failure with neuroendocrine activation can be induced by chronic ventricular tachypacing at a rate, which does not abolish the coronary reserve of left-ventricular subendocardial myocardium. Furthermore, we monitored left-ventricular diameters during tachypacing to exclude enhancement of load by diastolic ventricular distension. In this model, we studied whether myocardial ACE activity and angiotensinogen gene expression changed during the development of heart failure.

Methods

Animals

Male Wistar rats (Savo, Kisslegg, FRG) weighing 250–350 g were used in this study. They were fed standard chow and had free access to tap water. All protocols of the project were considered and approved by the ethics committee for animal welfare in science at Regierungspräsidium Freiburg. The care of the animals and the experimental procedures were supervised by an independent veterinarian in accordance with German laws and the animal welfare regulations of the University of Freiburg.

Instrumentation: The animals were anesthetized intraperitoneally with ketamine (30 mg) and xylazine (5 mg), then tracheotomized and placed on a ventilator. A thoracotomy was performed in the fourth left intercostal space, the pericardium was incised and two pacing electrodes were sewn on the apex of the ventricles. A pair of piezo-crystals (1.0-mm outer diameter including resin isolation, made in our laboratory) were sewn on the anterior and posterior epicardial surfaces of the left ventricle for measurement of the external diameter of the minor ventricular axis by the ultrasonic transit time analysis. After closure of thorax and trachea, the cables of electrodes and crystals were exteriorized at the neck and packed into a 1.2-cm long PVC tube (8-mm diameter), which was fixed to a leather collar.

The animals were allowed to recover from the operation for about 5 to 9 days, until they had regained their preoperative body weight and were in a phase of constant weight gain. Then the tube on the collar was connected to an elastic suspension system with a spiral spring. Within that spring, the cables were led to a swivel connector outside of the cage. Thus, the rat could move freely in its cage (26×40 cm), while acute or chronic registration from the electrodes or crystals was possible without disturbing the animal. The rats were allowed to adjust to this suspension system for 5 days. Their spontaneous heart rate (from ECG- or diameter recordings) was documented at least two times at the end of this period (days -2 and -1 in Table 1). Thereafter, ventricular tachypacing (610–620 impulses/min) or sham treatment without pacing was started (day 1 in table 1) and maintained for 2 weeks.

Overall, 126 rats were operated; 49 of them had exact function of the diameter registration (constant amplitude of the receiver signal, indicating parallel "en face" placement of the piezo-crystals) and were assigned to groups A or B (Table 1). The other rats (not used for the analysis of cardiac diameter) were assigned to groups C–H (Table 1) and received catheters implanted in anesthesia at various time-points during the protocols (Table 1). Chronic changes in the ratios of organ weight/body weight were not documented in these rats because of fluctuations in body weight after the implantation of catheters. The protocols applied in the various groups are summarized in Table 1.

Measurements

The enddiastolic external diameter of the left ventricle was obtained by the transit time analysis (4, 36) between the implanted crystals. Prior to the implantation, the piezocrystals were calibrated in vitro (from crystal-surface to the surface of the opposing crystal) by a micrometer caliper.

Table 1. Protocols.

Group and treatment	Early deaths (day 1–4)	Observations in vivo		Late deaths (day 5–14)	Analyses after sacrifice
A) n = 32 Tachypacing	n = 9	– diameter changes[a] and drinking behavior	n = 14 (inc. 2 late deaths)	n = 2	Tissue ACE n = 10
		– diameter recordings not completed due to cable defect	n = 9	none	Ventricular n = 9 A'gen mRNA
B) n = 17 Sham treatment	none	– diameter changes and drinking behavior	n = 10	none	Tissue ACE n = 6
		– diameter recordings not completed due to cable defect	n = 7	none	Ventricular n = 7 A'gen mRNA
C) n = 16 Tachypacing and cath. implantation[b] (day 12 or 13)	n = 5	– cardiac output and cardiopulmonary blood volume	n = 8 (2 deaths during cath. implantation)	n = 1	—
D) n = 10 Sham treatment and cath. implantation[b] (day 12 or 13)	none	– cardiac output and cardiopulmonary blood volume	n = 9 (1 death during cath. implantation)	none	—
E) n = 16 Tachypacing (4 days) cath. implantation[d] (day −2)	n = 6	– plasma hormones[c] (day −1 and day 4)	n = 8	—	—

Table 1. Protocols (continued).

Group and treatment	Early deaths (day 1–4)	Observations in vivo		Late deaths (day 5–14)	Analyses after sacrifice
F) n = 15 Tachypacing and cath. implantation[d] (day 12)	n = 5	– plasma hormones[c] (day 14)	n = 6 (3 deaths during cath. implantation)	n = 1	—
G) n = 7 Sham treatment and cath. implantation[d] (day 12)	none	– plasma hormones[c] (day 14)	n = 6 (1 death during cath. implantation)	none	—
H) n = 13 No chronic treatment cath. implantation[e] (day 0)	—	– coronary reserve (day 1)	n = 8 (3 deaths during cath. implantation 2 exclusions)	—	—

[a] diameters during pacing recorded every 2 to days; on these occasions, pacing was interrupted für 10 min and diameters were recorded with spontaneous heart rate, too.
[b] PE50 catheters in carotid artery and jugular vein.
[c] 1.5 ml arterial blood replaced by 2 ml dextran.
[d] PE50 catheter in femoral artery.
[e] PE50 catheters in jugular vein and left ventricle (via carotid artery) and PE50 catheter in femoral artery.

For measurement of cardiac output (CO) in heparinized rats (500 U/kg), we used a slightly modified dye dilution method (14): 1.25 mg of indiocyanine-green (0.1 ml) was injected via a central venous catheter and arterial blood was withdrawn (withdrawal rate: 5 ml/min) for 20 s from the carotid artery catheter through the cuvette of a densitometer. The cardiopulmonary blood volume (CPBV) was calculated from the mean transit time of the dye from the injection site (right atrium) to the aortic arch (carotid catheter) multiplied by CO (16). Estimates of CO and CPBV were obtained by averaging three consecutive measurements (with reinjection of the withdrawn blood after each measurement) and demonstrated a variability of 11 % and 8 %, respectively.

For the assessment of coronary reserve during tachypacing, the microsphere technique (13) was applied as described previously (10). Approximately 250 000 microspheres (^{103}Ru, ^{141}Ce, ^{95}Nb, diameter 15 μm, DuPont-NEN, Dreieich, FRG), suspended in 0.5 ml of 0.9 % NaCl and 0.01 % Tween 80 solution were injected during 15 s in the left ventricle, followed immediately by a 0.5-ml saline flush for 15 s. Blood was withdrawn beginning 15 s before the microspheres injection at a constant rate of 0.30 ml/min for a total withdrawal time of 2 min from the femoral artery catheter. Measurements were done at spontaneous heart rate (control) after 10 min of pacing (615–620 bpm) and after an additional 10 min with pacing and intravenous infusion of dipyridamole (2 mg/kg/min). Animals were killed by an overdose of anesthetic and organs (heart, kidneys) together with the reference blood sample were harvested for subsequent gamma counting. Regional blood flow and cardiac output were calculated by established formulas. Values of two animals were excluded because radioactivity in the lungs of more than 3 % of the injected activity was regarded as indicating shunt flow.

For biochemical measurements, the animals were sacrificed with an overdose of anesthetic. A 2-ml blood sample was quickly withdrawn by puncture of the aorta and the hearts were arrested with 0.9 % icecold saline. Hearts, lungs, kidneys, and aorta were blotted dry and immediately placed on ice for subsequent analyses of tissue angiotension converting enzyme (ACE) activity. Hearts for the determination of angiotensinogen mRNA were stored in liquid nitrogen.

The tissue ACE activity was determined with a modified method of Okamura et al. (35). After extraction and centrifugation at 20 000 g for 20 min, 50 μl of the supernatant were incubated at 37 °C for 1 or 3 h with 150 μl of sodium-phosphate buffer containing 23.2 mg of hippurylhistidyl-leucin (HHL) and 400 μl of radioactive labeled C-14 HHL (final concentration 5 mM). The rate of production of radioactive labeled hippuric acid from HHL was measured with a radiochemical assay by β-counting. ACE activity was expressed in nmol/min/mg protein. Angiotensinogen mRNA was measured with a quantitative solution hybridization assay as previously described (15). Total RNA was extracted from pooled left and right ventricles with the LiCl/urea method as described previously (15) and quantified photometrically at 260 nm after check of RNA integrity on ethidium bromide-stained agarose gels.

We determined in 0.8 ml arterial plasma the concentrations of norepinephrine (NE), epinephrine (EPI), and atrial natriuretic peptide (ANP), together with plasma renin activity (PRA) as described previously in dogs (17). Plasma arginine vasopressin (AVP) was measured from plasma after extraction over C-18 columns (recovery 90 %, interassay variability 5.5 %, detection limit 0.1 pg) with a commercially available AVP antibody (Amersham, Braunschweig, FRG).

Calculations

Values in this paper are given as means ± SEM. Significance of differences was estimated by *t*-test for paired or independent samples or by 2-way ANOVA with a subsequent Bonferroni *t*-test as appropriate; log-transformation was used for these tests if variances of groups differed. A value of $p < 0.05$ was considered significant.

Results

General observations

Chronic ventricular tachypacing was started in 79 rats (groups A, C, E and F, Table 1). Twenty-five of them (= 32 %) died within the first 4 days of pacing; four (= 6 %) died during the second week of pacing, yielding a 38 % mortality over 2 weeks (Table 1). In 34 sham rats (groups B, D and G) no deaths occurred.

During the 2 weeks of tachypacing, the increase in body weight of group A was $7.6 \pm 1.9\%$ ($n = 19$), while it was $5.4 \pm 1.4\%$ ($n = 17$) during the same period in the sham rats of group B. These increases were not significantly different. Mean drinking volume in group A was 102 ± 3 ml/kg/day, similar to 111 ± 7 ml/kg/day in group B.

After sacrifice, chronically paced rats had higher lung weight per body weight (5.9 ± 0.2 mg/g, group A, $n = 17$, vs 4.2 ± 0.1 mg/g, group B, $n = 13$, $p < 0.001$), while the dry weight of the lungs was similar in both groups ($14.7 \pm 0.3\%$ in group A, $14.9 \pm 0.9\%$ in group B). Ventricular weight (left + right) per body weight was not elevated in paced rats (2.69 ± 0.03 mg/g, group A, $n = 19$, vs 2.73 ± 0.08 mg/g, group B, $n = 17$; n.s.), but atrial weight per body weight was more than doubled (left + right atrium, 0.63 ± 0.04 mg/kg, group A, $n = 10$, vs 0.26 ± 0.02 mg/kg, group B, $n = 6$, $p < 0.01$). Atrial weights were obtained only from rats in which tissue ACE activity was measured (see below).

Ventricular dilation

In the sham-treated rats of group B, the external enddiastolic diameter increased significantly during the 2 weeks of observation, with an average increase of 32 ± 4 µm/day, while spontaneous heart rate remained unchanged (Fig. 1). One day after the onset of tachypacing in group A, enddiastolic ventricular diameter during pacing tachycardia was $2.6 \pm 0.7\%$ below the last control diameter on sinus rhythm ($p < 0.01$). It increased by 77 ± 13 µm/day during the 2 weeks of pacing, but did not exceed the enddiastolic diameter of the sham rats on sinus rhythm (Fig. 1). However, when the ventricular pacer was turned off

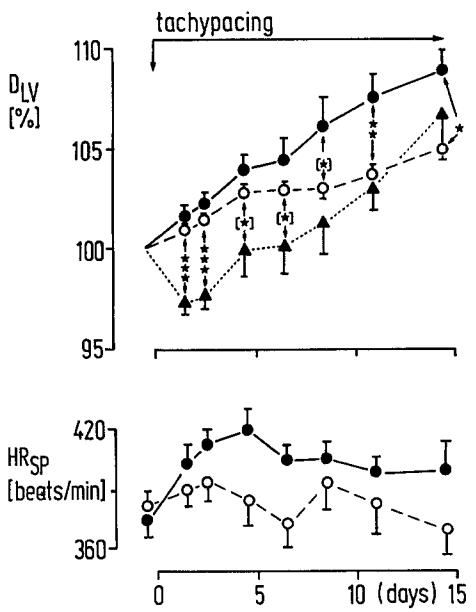

Fig. 1. Changes in relative left-ventricular enddiastolic external diameter (D_{LV}) in % of value on day -1, upper panel, and in spontaneous heart rate (HR_{SP}), lower panel. (●) Paced rats on sinus rhythm, when pacing was interrupted for 10 min ($n = 14$ initially, two rats died in the second week of pacing; values from rats dying within the first 4 days of tachypacing are not included); (▲) paced rats during tachypacing; (○) sham treated rats ($n = 10$); means and bars = SEM. (*) $01 > p > 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs sham.

for 10 min on each occasion of diameter recording, the rats resumed spontaneous sinus rhythm at a rate slightly above that of the sham rats (Fig. 1). Enddiastolic ventricular diameter during these short periods without pacing increased by 79 ± 9 µm/day ($p < 0.001$ compared to the increase of 32 ± 4 µm/day in rats of group B).

Hemodynamics

Chronically paced rats (group C) had lower cardiac output, stroke volume, and mean arterial pressure compared to sham rats (group D), but central venous pressure and cardiopulmonary blood volume were higher (Table 2). When the tachypacing was interrupted for 10 min, heart rate, cardiac output, and stroke volume of the chronically paced rats were normalized (Table 2). However, cardiopulmonary blood volume and central venous pressure still remained above the control values of sham rats, while mean arterial pressure and total peripheral vascular resistance were lower (Table 2).

Acute tachypacing for 10 min in sham rats did not lower cardiac output, but slightly elevated central venous pressure and cardiopulmonary blood volume (Table 2).

Cardiac ACE-activity and angiotensinogen expression

In chronically paced rats, atrial and ventricular ACE activities were significantly elevated compared to sham rats, while no differences were found in ACE activities of other peripheral tissues and of arterial plasma (Fig. 2). Ventricular concentration of angiotensino-

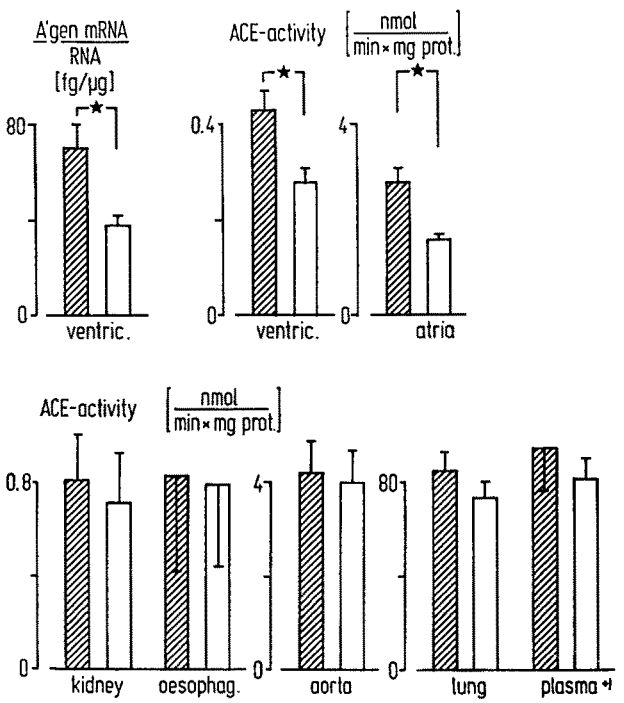

Fig. 2. Cardiac angiotensinogen gene expression (angiotensinogen messenger RNA/total RNA) and tissue angiotensin converting enzyme (ACE) activity in paced rats (shaded columns, n = 9–10) and in sham rats (open columns, n = 6–7). $^{+}$) plasma ACE activity is given in nmol · min^{-1} · ml^{-1}; * $p < 0.05$.

Table 2. Hemodynamic effects of acute and chronic tachypacing.

	Group D (sham treatment)		Group C (chronic tachypacing)		Difference of basal values
	Basal (sinus rhythm) n = 9	Pacing (10 min) n = 6	Basal (pacing) n = 8	Stop of pacing (10 min) n = 8	
HR (beats · min^{-1})	373 ± 20	618 ± 1	616 ± 1	350 ± 15	—
CO (ml · min^{-1} · kg^{-1})	349 ± 8	338 ± 11	301 ± 10	353 ± 12***	$p < 0.001$
SV (ml · kg^{-1})	0.97 ± 0.04	0.55 ± 0.02***	0.49 ± 0.02	1.02 ± 0.05***	$p < 0.001$
CPBV (ml · kg^{-1})	14.2 ± 0.6	14.8 ± 0.8**	18.4 ± 0.8	16.5 ± 0.4**	$p < 0.01$
CVP (mm Hg)	1.1 ± 0.4	2.8 ± 0.3*	3.6 ± 0.7	1.7 ± 0.2*	$p < 0.05$
MAP (mm Hg)	115 ± 4	103 ± 4(*)	103 ± 5	100 ± 6	$0.1 > p > 0.05$
PVR (kPa · l^{-1} · min · kg)	44 ± 2	41 ± 2(*)	46 ± 1	38 ± 2***	NS

HR = heart rate; CO = cardiac output; SV = stroke volume; CPBV = cardiopulmonary blood volume; CVP = central venous pressure; MAP = mean arterial pressure; PVR = peripheral vascular resistance; NS = not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; (*) $0.1 > p > 0.05$ vs respective basal value.

Table 3. Neuroendocrine activity.

	Group E (n = 8) prepacing (day −1)	pacing (day 4)	Group G (n = 6) sham (day 14)	Group F (n = 6) tachypacing (day 14)	Significance
ANP (pg · ml^{-1})	115 ± 22	220 ± 33**	165 ± 65	534 ± 123	$p < 0.005$
AVP (pg · ml^{-1})	1.05 ± 0.35	2.54 ± 0.20	0.94 ± 0.21	3.08 ± 0.59	$p < 0.001$
NE (pg · ml^{-1})	452 ± 60	1260 ± 240**	361 ± 77	485 ± 48	NS
Epi (pg · ml^{-1})	252 ± 41	980 ± 282**	215 ± 65	450 ± 103	$0.1 > p > 0.05$
PRA (ng · ml^{-1} · min^{-1})	3.2 ± 0.4	5.9 ± 0.6*	4.2 ± 0.6	3.9 ± 0.2	NS
MAP (mm Hg)	109 ± 2	88 ± 2**	112 ± 4	97 ± 6	$0.1 > p > 0.05$
HR (beats · min^{-1})	372 ± 19	615 ± 1	381 ± 12	617 ± 1	—

ANP = atrial natriuretic peptide; AVP = arginine vasopressin; NE = norepinephrine; Epi = epinephrine; PRA = plasma renin activity; MAP = mean arterial pressure; HR = heart rate; NS = not significant; significance refers to the effect of chronic tachypacing (column 4) vs sham treatment (column 1); * $p < 0.05$; ** $p < 0.01$ vs prepacing.

gen-mRNA per total RNA was doubled in chronically paced rats compared to sham rats (Fig. 2), while total RNA content per ventricular wet weight was similar (0.96 ± 0.07 µg/mg in sham rats and 1.00 ± 0.03 µg/mg in chronically paced rats). In another pilot series, ventricular A'gen-mRNA/total RNA was 73 ± 13 fg/µg ($n = 14$) in rats after 4 days of tachypacing and 374 ± 174 fg/µg ($n = 4$) in rats after 2 weeks of tachypacing, but only 52 ± 6 fg/µg ($n = 10$) in sham rats. The tachypaced rats of this pilot series were more frequently stressed, because pacing equipment had to be repaired. Therefore, effects of tachypacing and of stress could not be clearly separated.

Atrial ACE activity and relative atrial weight correlated closely with lung weight per body weight (Fig. 3). This latter parameter probably reflects the degree of chronic pulmonary congestion in the chronically paced rats. Ventricular dilation, i.e., increase of enddiastolic

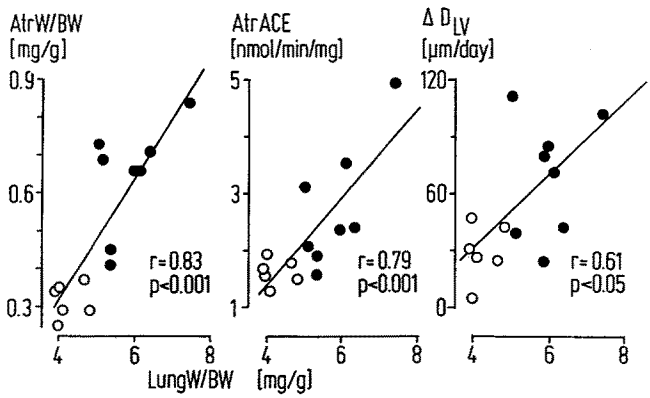

Fig. 3. Correlations of lung weight per body weight (LungW/BW) to relative atrial weight (AtrW/BW), to atrial angiotensin converting enzyme activity (AtrACE) and to ventricular dilation (i.e., daily increase in enddiastolic external left-ventricular diameter under sinus rhythm). (●) paced rats, (○) sham rats.

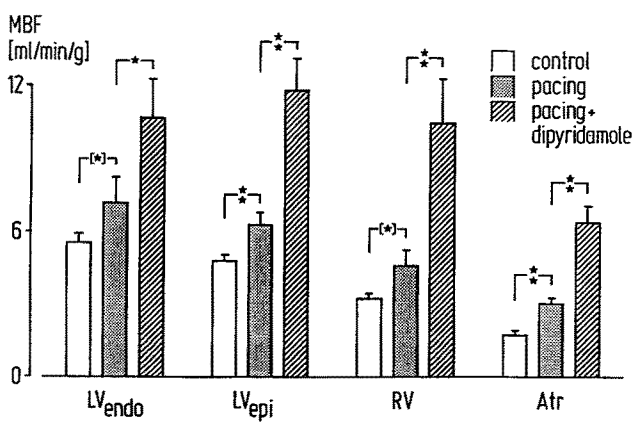

Fig. 4. Regional myocardial blood flow (MBF) in left-ventricular subendocardium (LV_{endo}), left-ventricular subepicardium (LV_{epi}), free wall of right ventricle (RV) and left and right atrium (Atr). (*) $0.1 > p > 0.05$; * $p < 0.05$; ** $p < 0.01$.

ventricular diameter per day during spontaneous heart rate (Fig. 1), also correlated positively to lung weight per body weight (Fig. 3), while no such correlation existed for ventricular ACE activity or ventricular angiotensinogen gene expression.

Neuroendocrine activity

Four days after the onset of tachypacing, catecholamines, renin activity, arginine-vasopressin, and atrial natriuretic peptide in arterial plasma were significantly increased compared to the prepacing control value (Table 3). However, in rats surviving 2 weeks of tachypacing, catecholamines and renin activity had returned close to the values of unpaced sham rats, while atrial natriuretic peptide and arginine vasopressin remained elevated (Table 3).

Coronary reserve during tachypacing

Values of myocardial blood flow during assessment of coronary reserve are shown in Fig. 4, hemodynamic values obtained during these assessments are given in Table 4. Though dipyridamole infusion in rats under acute tachypacing lowered mean arterial pressure (Table 4), it significantly increased regional myocardial blood flow in all regions of the heart. In the subendocardial layers of the left ventricle, this dipyridamole-induced increase still amounted to $56 \pm 15\,\%$ (Fig. 4).

Discussion

In the rats subjected to chronic ventricular tachypacing, we observed typical features of congestive heart failure: low cardiac output, enhanced cardiopulmonary blood volume and neuroendocrine activation (Tables 2 and 3). In view of a 30 % higher cardiopulmonary blood volume, the 14 % decrease in cardiac output (Table 2) must reflect some degree of chronic cardiac deterioration, since the acute effects of tachypacing on both parameters are neglegible (Table 2, group D, and Table 4). While the recordings of ventricular diameter during pacing as well as during sinus rhythm indicated ongoing cardiac enlargement in these chronically paced rats (Fig. 1), their ventricular mass was not augmented. Thus, in the rats surviving 2 weeks of tachypacing, slowly ongoing ventricular dilation with marginal functional impairment developed, qualitatively comparable to the chronic alterations reported in the canine tachypacing model (3, 19, 47), but much less severe in extent.

Table 4. Hemodynamics during assessment of coronary reserve (group H).

	Basal n = 8	Pacing (10 min) n = 8	Pacing (20 min) and dipyridamole (10 min) n = 8
HR (beats · min^{-1})	395 ± 15	618 ± 1	618 ± 1
CO (ml · min^{-1} · kg^{-1})	355 ± 16	345 ± 25	400 ± 25
SV (ml · kg^{-1})	0.93 ± 0.07	0.56 ± 0.04	0.65 ± 0.04
MAP (mm Hg)	115 ± 5	110 ± 6	97 ± 5**
PVR (kPa · l^{-1} · min · kg)	43 ± 3	43 ± 5	33 ± 3*
RBF (ml · min^{-1} · g^{-1})	5.2 ± 0.6	4.7 ± 0.4	4.8 ± 0.4

HR = heart rate; CO = cardiac output; SV = stroke volume; MAP = mean arterial pressure; PVR = peripheral vascular resistance; RBF = renal blood flow; * $p < 0.05$; ** $p < 0.01$ vs pacing.
Pacing significantly lowered MAP ($p < 0.01$) and SV ($p < 0.001$) vs basal values.

Mechanical overload or myocardial hypoperfusion cannot have initiated these alterations, since arterial pressure and left-ventricular external diameter during tachypacing were initially lower and never exceeded the respective values of the sham animals (Fig. 1). Therefore, enhanced wall stress during contraction did not occur. Under acute tachypacing, dipyridamole still induced a significant increase in the left-ventricular subendocardial blood flow (Fig. 4), despite a substantial fall in blood pressure (Table 4). Thus, subendocardial ischemia during tachypacing appears unlikely. This load- and ischemia-independent model of heart failure with initially elevated neuroendocrine activity and subsequently developing cardiac impairment may serve as a model to further analyze the potentially cardiotoxic effects of chronically elevated neuroendocrine activity.

Chronic depression of cardiac output is much more pronounced in dogs on tachypacing (3, 19, 30, 37, 38, 46) than with the rats studied here. The endocrine changes in the canine model are progressing with time and are generally larger (19, 32, 37, 38), while in the rats some parameters of neuroendocrine activation tend to normalize again after 2 weeks (Table 3, group F). On the other hand, we observed a substantial mortality during the first 4 days of tachypacing (Table 1), which is not reported in the canine model. Some of the differences in comparison to the canine model may be due to the heterogeneity within the group of chronically instrumented rats. A variable degree of ventricular necrosis was probably induced by suturing the piezo-crystals to the ventricular surface of these small hearts. We may have lost the animals with the more damaged ventricles during the first days of pacing, while for the other rats, the chronic tachypacing with 615 impulses per minute caused only a modest impairment which could be well compensated. Accordingly, we found no ascites and only a moderate elevation in the wet weight of the lungs.

This model revealed elevated expression of the angiotensinogen gene and enhanced ACE-activity in ventricular myocardium (Fig. 2). Similarly, modest activations of the local cardiac RAS have been reported in rats with pressure overload (40) or with myocardial infarction (11). Our data in the pilot series, however, indicate that ventricular angiotensinogen gene expression in the rat can be more greatly augmented.

Neuroendocrine activity may act as stimulus of the tissue RAS: Angiotensin-II stimulates hepatic angiotensinogen gene expression (24), β-adrenergic activation of local angiotensin-II formation in nonrenal vascular tissue has been demonstrated (31, 39, 44) and chronic changes in local ACE-activity in peripheral tissues (arteries, lung) occur in renal hypertension with transient activation of circulating renin activity (35). However, in our model, the increase in local ACE-activity occurred only in the heart (Fig. 2). This organ-selective response can hardly be explained by systemic neuroendocrine activation.

Altered local cardiac contractile activation and neuroendocrine effects may act synergistically to induce changes of the cardiac RAS. In the rats with myocardial infarction, enhanced myocardial wall stress was considered a stimulus for the activation of the cardiac RAS (11). Enhanced atrial wall stress can be assumed in our rats with chronic ventricular tachypacing and cardiopulmonary overfilling and may be the explanation for the observed correlation of relative lung weight to atrial ACE activity and atrial weight (Fig. 3). In the ventricles, however, myocardial wall stress cannot be elevated during tachypacing, but the electrically induced excitation of contraction could play a permissive role.

The rise in local myocardial angiotensinogen expression and ACE activity together with the transient elevation in systemic circulating renin activity indicate that cardiac angiotensin-II formation may be increased during chronic tachypacing. Several observations support this assumption: first, kinetic analyses of plasma angiotensins demonstrated that the major part of circulating angiotensins is formed locally in peripheral tissues (2, 5, 6); secondly, the ACE-activity appears to be the rate-limiting step for this local synthesis of circulating angiotensin-II (7), though other angiotensin-forming enzymes may contribute (12); thirdly,

enhanced intracardiac conversion of angiotensin I to angiotensin II in isolated, perfused rat hearts with hypertrophy (and with an elevated ventricular ACE activity comparably to the tachypaced hearts; Fig. 2) causes dose-dependent coronary constriction and depression of diastolic ventricular relaxation (40); finally, local angiotensin II synthesis largely depends on the delivery of circulating active renin from the kidneys (7, 8, 21, 42), which may contribute to the local synthesis after uptake and accumulation in the tissues (28, 45).

The contribution of local angiotensin synthesis from the heart or from other organs cannot yet be quantified in our experiments. However, angiotensin formation from cardiac angiotensinogen has been demonstrated recently in isolated perfused rat hearts (26). The site of angiotensin synthesis must be important for potential local autocrine or paracrine effects (40) of the newly formed angiotensin-II prior to its release into the circulation. The potentially pathophysiologic role of enhanced local angiotensin II formation – acutely on coronary flow, sympathetic transmission and diastolic function, and/or chronically on cardiac growth and myocardial phenotype – is the subject of attractive, but still unsubstantiated hypotheses. The enhancement of the cardiac RAS in the present study has to be seen in context with our recent observation of a preventive action of low-dose ACE-inhibition in the canine tachypacing model (18). In dogs on tachypacing, chronic ACE inhibition was applied at a dosage that did not lower arterial pressure or peripheral vascular resistance. Without hemodynamic unloading, this treatment retarded the decline in cardiac output and attenuated systemic neuroendocrine activity (18). Whether a modulation of the cardiac RAS contributed to the load-independent preventive effect of this treatment in the canine tachypacing model or in other forms of cardiac failure deserves further study.

In conclusion, tachypacing in rats at a rate of 610 to 620 per minute does not abolish the coronary dilatory reserve in left-ventricular subendocardial myocardium and cannot elevate ventricular wall stress. However, when applied chronically it induces moderate, progressive low-output failure with neuroendocrine activation and with enhanced cardiac ACE activity and angiotensinogen expression.

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