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Effects of quinapril, losartan and hydralazine on cardiac hypertrophy and β-adrenergic neuroeffector mechanisms in transgenic (mREN2)27 rats

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1 Desensitization of the myocardial β-adrenergic signal transduction pathway is an important mechanism which is involved in the progression of hypertensive heart disease. The aim of the present study was to evaluate the differential effects of chronic pharmacotherapy with an angiotensin converting enzyme (ACE)-inhibitor, an AT_1-receptor antagonist and a direct vasodilator on blood pressure, cardiac hypertrophy and the β-adrenergic signal transduction. Therefore, transgenic TG(mREN2)27 (TG) rats overexpressing the mouse renin gene were used. This strain is characterized by the development of fulminant hypertension with cardiac hypertrophy.

2 Seven week old heterozygous TG(mREN2)27 rats were treated for 11 weeks with the AT_1-receptor antagonist losartan (10 mg kg^{-1}), the ACE-inhibitor quinapril (15 mg kg^{-1}) and the direct vasodilator hydralazine (30 mg kg^{-1}). Untreated TG and normotensive Sprague-Dawley rats (SD) served as controls.

3 TG(mREN2)27-rats were characterized by arterial hypertension (TG 194±3.2 mmHg vs SD 136±2.9 mmHg systolic blood pressure), increased left ventricular weights (TG 4.3±0.3 vs SD 3.0±0.1 mg g^{-1} body weight), decreased myocardial neuropeptide Y (NPY) concentrations (TG 1143±108 vs SD 1953±134 pg g^{-1} wet weight), reduced β-adrenoceptor densities (TG 51.1±1.9 vs SD 63.4±3.7 fmol mg^{-1}) as assessed by [^{125}I]-cyanopindolol binding studies, and increased $G_{i\alpha}$-activities (TG 4151±181 vs SD 3169±130 densitometric units) as assessed by pertussis toxin catalyzed [^{32}P]-ADP-ribosylation. Downregulation of β-adrenoceptors and increased $G_{i\alpha}$ were accompanied by significantly reduced isoprenaline-, Gpp(NH)p- and forskolin-stimulated adenylyl cyclase activity. Catalyst activity as determined by forskolin plus Mn^{2+} co-stimulation of adenylyl cyclase did not differ between TG(mREN2)27- and SD control-rats.

4 Losartan and quinapril significantly restored systolic blood pressures, left ventricular weights, β-adrenoceptor densities, myocardial neuropeptide Y-concentrations, adenylyl cyclase activities and $G_{i\alpha}$-activities towards the values in Sprague-Dawley-controls. No differences were observed between the effects of quinapril- and losartan-treatment. In contrast, hydralazine had only minor effects on blood pressure reduction, regression of left ventricular hypertrophy and neuroeffector defects in TG(mREN2)27.

5 In conclusion, direct vasodilatation is not able to overcome the pathophysiological alterations in TG caused by transgene overexpression. In contrast, ACE-inhibitors and AT_1-receptor antagonists, which inhibit the renin angiotensin system, equally exert beneficial effects on blood pressure, myocardial hypertrophy and neuroeffector mechanisms. Modulation of the sympathetic tone and resensitization of the β-adrenergic signal transduction system may contribute to the special effectiveness of these drugs in the treatment of the hypertensive cardiomyopathy.

Keywords: Renin transgenic rat; adenylyl cyclase; G-protein; ACE-inhibitor; angiotensin II antagonist

Introduction

Arterial hypertension is the most common cause of the development of heart failure (Kannel *et al*., 1972). An enhanced activity of the sympathetic nervous system (Goldstein, 1983; Goldstein & Kopin, 1990) and an activation of the renin angiotensin system (RAS) (Miyazaki *et al*., 1987; for review Paran *et al*., 1995) which interacts with the sympathetic nervous system at different levels (Schwieler & Hjemdahl, 1992; Ganten, 1993), have been suggested to play a major role in the pathological process that leads from hypertension to heart failure. However, to date, the precise mechanisms involved are still a subject of speculation. Angiotensin II and catecholamines may exert trophic effects leading to structural changes of the hypertensive myocardium (Simpson, 1983; Laragh & Sealey, 1990; Weber & Brilla, 1992). Furthermore, desensitization of the β-adrenergic signal transduction system, which is associated with a subsensitivity of the heart to β-adrenoceptor-induced increase of inotropy (Agabiti Rosei *et al*., 1984; Hausdorff *et al*., 1990), may play a crucial role. At a cellular level, this alteration is due to a downregulation of β-adrenoceptors and an increased activity of inhibitory guanine nucleotide binding proteins ($G_{i\alpha}$) as a response to elevated catecholamine levels.

Blockade of the RAS has been found to be an effective treatment of hypertensive heart disease, probably better than any other antihypertensive medication. Although most vasodilators can successfully reduce blood pressure, clinical studies suggest medication that lowers neurohumoral activity,

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like ACE-inhibitors, to be more efficious at reducing left ventricular mass and inhibiting disease progression (Dahlof & Hansson, 1992; Francis *et al*., 1993) than therapeutic approaches that do not alter or even increase neuroendocrine activity, like the direct vasodilator hydralazine. However, to date, it is still unclear whether therapy with the novel class of selective angiotensin II subtype 1 receptor antagonists is superior to ACE inhibition. Findings from animal studies comparing the effects on left ventricular hypertrophy remain controversial. AT_1-receptor antagonism has been shown to be inferior, superior or equivalent to ACE-inhibition (Linz *et al*., 1991; Ruzicka *et al*., 1993; Bruckschlegel *et al*., 1995). Until now, less is known about the effects of AT_1 receptor antagonists on the β-adrenoceptor-G-protein-adenylyl cyclase system. Since desensitization of the β-adrenergic signalling pathway is a putative mechanism which is involved in the development of heart failure, it is of interest to evaluate the differential effects of AT_1 receptor antagonists and ACE-inhibitors and direct vasodilators on β-adrenergic signal transduction, myocardial hypertrophy and sympathetic nervous tone in hypertensive cardiomyopathy. Therefore, we used a transgenic (mREN2)27 rat model with overexpression of the mouse renin 2^d gene. The resulting strain exhibits severe hypertension and cardiac hypertrophy (Mullins *et al*., 1990). The transgene is expressed at high levels in the adrenal, thymus, small intestine, testis, ovary, kidney, brain as well as in blood vessels and in the myocardium (Mullins *et al*., 1990). Although plasma prorenin is markedly increased in heterozygous TG(mREN2)27 animals, these rats have suppressed plasma renin and immunoreactive angiotensin levels (Mullins *et al*., 1990). This leads to the hypothesis that this monogenic form of hypertension is due to increased angiotensin peptide formation in specific tissue compartments like the vasculature or the myocardium. The possible pathophysiological role of the tissue RAS in transgenic rats parallels findings from animal models (supravalvular aortic stenosis, spontaneously hypertensive rats) (Li *et al*., 1989; Nagano *et al*., 1990; Schunkert *et al*., 1990) and man (aortic stenosis) (Studer *et al*., 1994) indicating an increased cardiac angiotensin II formation in the condition of pressure overload.

Methods

Animals and treatment

The animals in this study were handled in accordance with the guidelines of animal care of The Netherlands. Heterozygous transgenic TG(mREN2)27 animals and Sprague-Dawley (SD) control rats were housed and treated in the animal laboratory of the Department of Clinical Pharmacology, University of Groningen, The Netherlands. Animals were obtained from Møllegard (Denmark). TG(mREN2)27 and SD rats were maintained in a room kept at 25°C with 12 h light-dark cycle. The animals were kept on a standard laboratory animal diet and tap water *ad libitum*. SD rats were the animals into which the transgene was originally introduced. Treatment was started at the age of 7 weeks for 11 weeks. The drugs were administered orally. The average daily dose amounted to 15 mg kg^{-1} for quinapril, 10 mg kg^{-1} for losartan and 30 mg kg^{-1} for hydralazine. Systolic and diastolic blood pressures were measured every week by the tail-cuff method according to Pfeffer *et al*. (1971). At the end of the study, all animals were weighed and killed by cervical dislocation. The hearts were rapidly removed, washed with saline, blotted dry with filter paper, weighed and immediately frozen in liquid nitrogen. Samples were stored at −80°C. Hearts of TG(mREN2)27 exhibited concentric hypertrophy but no dilatation. No signs of venous congestion were observed in any other organ.

Membrane preparation

Myocardial tissue was chilled in 30 ml ice-cold lysis buffer (10 $mmol^{-1}$ Tris-HCl pH 7.4, 1 $mmol^{-1}$ EDTA, 1 $mmol^{-1}$ dithiothreiotol, 5 $\mu g\ ml^{-1}$ aprotinin, 5 $\mu g\ ml^{-1}$ leupeptin) and homogenized with a motor driven glass-teflon homogenizer in lysis buffer for 1 min. The homogenate was spun at 484 *g* (rotor Beckman JA 20) for 10 min to remove cellular debris and nuclei. The supernatant was filtered through two layers of cheese cloth and an equal volume of ice-cold 1 mmol l^{-1} KCl was added to extract contractile proteins, followed by stirring at 4°C for 10 min. This suspension was centrifuged at 100,000 *g* (rotor Beckman Ti 60) for 30 min. For radioligand binding experiments, the pellet was resuspended in 50 volumes of incubation buffer (50 mmol l^{-1} Tris-HCl, 10 mmol l^{-1} $MgCl_2$, pH 7.4) and recentrifuged at 100,000 *g* for 45 min. The final pellet was resuspended in incubation buffer (50 volumes) and was stored at −80°C. Storage did not alter the results. The protein concentration was determined according to Bradford (1976), with bovine IgG as standard.

Adenylyl cyclase determination

Adenylyl cyclase was determined according to Salomon *et al*. (1974). The reaction mixture containing 50 mmol l^{-1} [^{32}P]-α-ATP (approximately 0.3 μCi 100 ml^{-1}), 50 μmol l^{-1} triethanolamine-HCl, 5 mmol l^{-1} $MgCl_2$, 100 μmol l^{-1} EGTA, 1 mmol l^{-1} 3-isobutyl-1-methylxanthine (IBMX), 5 mmol l^{-1} creatine phosphate, 0.4 mg ml^{-1} creatine kinase, and 0.1 mmol l^{-1} adenosine 3′:5′-cyclic monophosphate (cyclic AMP) at pH 7.4 in a final volume of 100 μl was preincubated for 5 min at 37°C. The reaction was started by the addition of membrane (20 μg protein) and terminated by the addition of 100 μl stopping solution (40 mmol l^{-1} ATP, 14 mmol l^{-1} GTP, 2% (w/v) SDS). Separation of the reaction product [^{32}P]-cyclic AMP was achieved by sequential chromatography on Dowex 50 cation exchange and on neutral aluminium columns. The ^{32}P counts were corrected for column recovery (70–80%) by using [^{3}H]-cyclic AMP. The assay was linear with regard to time and protein concentration. Adenylyl cyclase activities were studied under basal conditions and in the presence of 10 μmol l^{-1} isoprenaline, 100 μmol l^{-1} Gpp(NH)p, 100 μmol l^{-1} forskolin, or 5 mmol l^{-1} $MnCl_2$. In experiments with $MnCl_2$, $MgCl_2$ was withheld from the assay.

Radioligand binding studies

Assays were performed in a total volume of 250 μl incubation buffer (50 mmol l^{-1} Tris/HCl, 10 mmol l^{-1} $MgCl_2$, pH 7.4). The incubation was carried out at 37°C for 60 min. These conditions allowed complete equilibration of the receptors with the radioligand. The reaction was terminated by rapid vacuum filtration through Whatman GF/C filters, and filters were immediately washed three times with 6 ml of ice-cold buffer (50 mmol l^{-1} Tris/HCl, 10 mmol l^{-1} $MgCl_2$, pH 7.4). Myocardial β-adrenoceptors were studied with [^{125}I]-cyanopindolol (ICYP, specific activity: 2000 Ci $mmol^{-1}$). Specific binding was determined by subtracting the amount of nonspecific binding measured in the presence of 3 μmol l^{-1} (−)-propranolol from the total binding. The maximum

number of binding sites was calculated by Scatchard anlaysis from saturation experiments.

Pertussis toxin-catalyzed [^{32}P]-ADP ribosylation

[^{32}P]-ADP ribosylation of $G_{i\alpha}$ by pertussis toxin was performed for 18 h at 4°C in a volume of 50 μl containing 100 mmol l^{-1} Tris/HCl (pH 8.0), 25 mmol l^{-1} dithiothreitol, 2 mmol l^{-1} ATP, 1 mmol l^{-1} GTP, 50 nmol l^{-1} [^{32}P]-NAD (800 Ci mmol^{-1}), Lubrol PX 0.5% (v/v), and 20 μg ml^{-1} pertussis toxin that had been activated by incubation with 50 mmol l^{-1} dithiothreitol for 1 h at 24°C before the labelling reaction. Samples were subjected to SDS–PAGE (10% w/v acrylamide, 16 cm total gel length) according to Laemmli (1970). Gels were stained with Coomassie blue and dried before autoradiography was performed. Incorporated [^{32}P]-ADP-ribose was quantified by cutting out the bands and measuring the radioactivity with a scintillation counter (Beckman LS 6500, Fullerton, U.S.A.).

Neuropeptide Y determinations

For neuropeptide Y measurements, tissue samples were homogenized with a Polytron device in TED-buffer (20 mmol l^{-1} Tris-HCl pH 7.4, 1 mmol l^{-1} EDTA, 1 mmol l^{-1} dithiothreitol, 5 μg ml^{-1} aprotinin, 5 μg ml^{-1} leupeptin). The homogenate was centrifuged at 100,000 *g* for 30 min and neuropeptide Y was determined in the supernatant with a commercially available radioimmunoassay (Immunodiagnostik, Bensheim, Germany).

Materials

GTP, guanylylimidodiphosphate (Gpp(NH)p), ATP, creatine phosphate and creatine kinase were purchased from Boehringer-Mannheim (Germany). Isobutylmethylxanthine (IBMX) (±)-isoprenaline, pertussis toxin were obtained from Sigma. [^{32}P]-ATP, [^{32}P]-NAD and the ligand [^{125}I]cyanopindolol were from Amersham Buchler (Braunschweig, Germany). All compounds used were of analytical or best grade commercially available. Only deionized and double distilled water was used throughout.

Statistics

Data are presented as mean ± s.e.mean. Statistical significance was estimated with Student's *t* test for unpaired observations and ANOVA according to Wallenstein *et al*., (1980). A probability value less than 0.5 was considered significant.

Results

Blood pressure and heart weights

Cardiac morphological and functional parameters are summarized in Table 1. The ratio of left ventricular weight to body weight was significantly elevated by about 40% in TG(mREN2)27 compared to SD. Treatment of TG(mREN2)27 with quinapril and losartan significantly reduced the relative left ventricular weight below the values observed in SD-controls, whereas the decrease of relative left ventricular weight after hydralazine-administration was about 15% and did not reach the level of SD-controls. The ratio of right ventricular weight to body weight was significantly reduced in the losartan- and quinapril-treated group whereas no differences were observed between SD-, hydralazine- and untreated TG(mREN2)27-rats. Systolic blood pressures were similarly reduced after quinapril- and losartan-treatment by about 30% and did not significantly differ from values of SD-controls. Hydralazine did significantly lower blood pressure but not by as much as quinapril or losartan. Heart rates were significantly increased in TG(mREN2)27 compared to SD-controls. Hydralazine treatment further increased heart rates whereas quinapril- and losartan treatment had no significant effect in TG(mREN2)27 compared to untreated transgenic control rats.

Neuropeptide Y concentration

In order to evaluate the degree of cardiac sympathetic activation, neuropeptide Y concentrations in left ventricular myocardium from TG(mREN2)27, SD-controls and TG(mREN2)27 treated with quinapril, hydralazine and losartan were determined. As shown in Figure 1, NPY-levels were significantly reduced by 40% in TG(mREN2)27, whereas ACE-blockade with quinapril and AT_1-receptor blockade with losartan completely restored the concentration of NPY in the myocardium, similar to values of SD. Hydralazine-treatment did not significantly change the NPY-level compared with untreated TG(mREN2)27.

β-Adrenoceptors

To address the question whether activation of the cardiac sympathetic nervous system is associated with alterations of β-adrenoceptor density, we performed saturation binding experiments with [^{125}I]-cyanopindolol in left ventricular membrane preparations from TG(mREN2)27, SD-controls

Table 1 Body and heart weights, heart rates (measured at the end of the treatment period), mean systolic blood pressures (measured after 5 and 11 weeks of treatment, respectively) and mean diastolic blood pressures (measured after 5 weeks of treatment) in SD control rats and TG(mREN2)27 rats treated with hydralazine, quinapril, losartan or kept under control conditions

		TG(mREN2)27			
	SD ($n=12$)	*Control* ($n=12$)	*Hydr* ($n=14$)	*Quin* ($n=12$)	*Los* ($n=12$)
Body weight (g)	483 ± 9	492 ± 33	497 ± 17	508 ± 10	558 ± 9*
LV weight/body weight (mg g^{-1})	3.0 ± 0.1	4.3 ± 0.3*	3.6 ± 0.1*#	2.6 ± 0.1*#	2.6 ± 0.1*#
RV weight/body weight (mg g^{-1})	0.79 ± 0.03	0.82 ± 0.05	0.80 ± 0.03	0.69 ± 0.02*#	0.67 ± 0.01*#
Heart rate (beats min^{-1}) (11 weeks)	361 ± 6.4	405 ± 6.6*	429 ± 6.9*#	404 ± 10.5*	385 ± 12.0
SBP (mmHg) (during treatment)	112 ± 3.5	172 ± 3.4*	155 ± 3.3*#	114 ± 2.8#	113 ± 2.4#
SBP (mmHg) (11 weeks)	136 ± 2.9	194 ± 3.2*	176 ± 3.1*#	136 ± 3.2#	136 ± 2.1#
DBP (mmHg) (during treatment)	112 ± 3.6	172 ± 3.4*	155 ± 3.3*#	114 ± 2.8#	113 ± 2.4#

Values are expressed as means ± s.e.mean. LV, left ventricular; SBP, systolic blood pressure; DBP, diastolic blood pressure; Hydr, hydralazine; Quin, quinapril; Los, losartan. *$P<0.05$ vs Sprague-Dawley control-rats; #$P<0.05$ vs TG(mREN2)27 control-rats.

and TG(mREN2)27 treated with quinapril, hydralazine and losartan. As shown in Figure 2, radioligand binding experiments demonstrated a decrease in β-adrenoceptor density of approximately 20% in left ventricles of TG(mREN2)27 compared to controls. Treatment with both quinapril and losartan significantly increased β-adrenoceptor number to the level of SD-controls, whereas hydralazine treatment did not affect β-adrenoceptor density in TG(mREN2)27. Mean K_D-values did not differ significantly between the treatment and control groups (SD: 46 pmol l^{-1}, $n=10$; TG(mREN2)27: 47 pmol l^{-1}, $n=10$).

Adenylyl cyclase activity

To study whether different pharmacological treatment regimens, altered sympathetic activity and altered β-adrenoceptor function have an impact on cyclic AMP accumulation, we investigated adenylyl cyclase activities under various stimulation conditions. The data are summarized in Figures 3 and 4. Basal adenylyl cyclase activities (in the presence of 10 μmol l^{-1} GTP) as well as the effects of the β-agonist isoprenaline, the nonhydrolyzable GTP-analogue Gpp(NH)p and the diterpen derivate forskolin on cyclic AMP-formation were significantly reduced in left ventricular membranes from TG(mREN2)27 compared to SD-controls. Treatment with hydralazine, quinapril and losartan restored adenylyl cyclase activities in TG(mREN2)27, whereas hydralazine treatment failed to normalize isoprenaline- and Gpp(NH)p-stimulated adenylyl cyclase activities. In order to investigate whether altered function of the catalyst of adenylyl cyclase could play a role, enzyme activity was stimulated in the presence of manganese ions and absence of magnesium. Under these conditions, the catalyst activity is independent of the influence of GTP-activated G-proteins (Cech *et al*., 1980; Cech & Maguire, 1982). No significant differences in $MnCl_2$ plus forskolin-stimulated cyclic AMP-formations were observed.

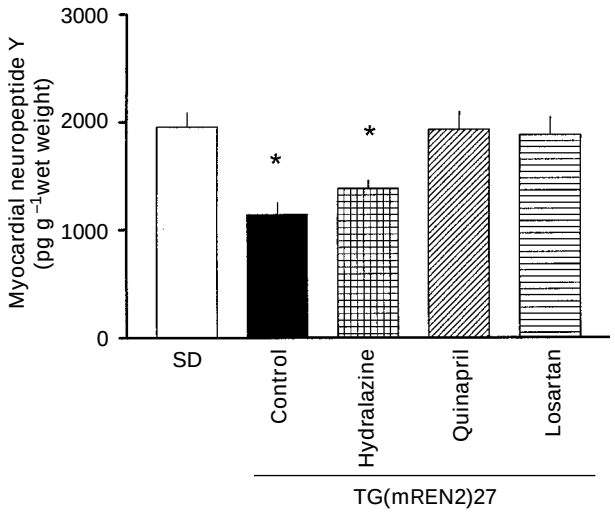

Figure 1 Left ventricular neuropeptide Y-concentrations in Sprague-Dawley control rats (SD) and transgenic rats (TG(mREN2)27) treated with hydralazine, quinapril, losartan or kept under control conditions. Values are means and vertical lines show s.e.mean ($n=8$). *$P<0.05$ vs corresponding values in Sprague-Dawley rats.

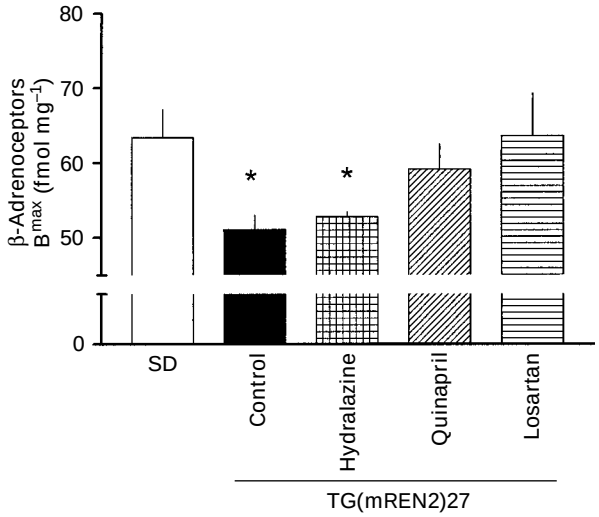

Figure 2 Density of β-adrenoceptors in left ventricles in Sprague-Dawley control rats (SD) and transgenic rats (TG(mREN2)27) treated with hydralazine, quinapril, losartan or kept under control conditions. Each column represents mean data ($n=8$) and vertical lines show s.e.mean. *$P<0.05$ vs corresponding values in Sprague-Dawley rats.

Inhibitory G-protein α-subunits

Alterations of inhibitory G-protein ($G_{i\alpha}$) function or content is well known to be one mechanism of adenylyl cyclase desensitization in heart failure. In order to assess $G_{i\alpha}$-activity, pertussis toxin catalyzed [^{32}P]-ADP-ribosylation experiments were carried out in left ventricular membrane preparations (Figure 5). The amount of incorporated [^{32}P]-ADP-ribose was significantly increased, by about 30%, in TG(mREN2)27 compared to controls. Hydralazine-treatment did not significantly reduce $G_{i\alpha}$-activity while quinapril and losartan administration restored $G_{i\alpha}$-activity.

Discussion

Vasodilator treatment with quinapril, losartan and hydralazine was performed in heterozygous transgenic rats, carrying the murine Ren2^d-gene, which were characterized by severe hypertension with cardiac hypertrophy. Blood pressure was reduced both by quinapril and losartan towards values in Sprague-Dawley (SD)-controls. This is not unexpected since hypertension is due to an activation of the (tissue) RAS in this monogenic model (Mullins *et al*., 1990; Ganten *et al*., 1991;Arribas *et al*., 1994). However, treatment with hydralazine failed to reduce blood pressure effectively, although even lower doses of hydralazine are sufficient to normalize blood pressure in other genetic or experimental rat models (Tsoporis & Leenen, 1988; Bruckschlegel *et al*., 1995). One possible explanation may be a much more pronounced local generation of angiotensin II (Hilgers *et al*., 1992) and endothelian (Gardiner *et al*., 1995) in transgenic (mREN2)27 rats, influencing the vascular tone in an autocrine/paracrine fashion. Furthermore, the locally synthesized angiotensin II potentiates the vascular reactivity to noradrenaline (Clough *et al*., 1982; Arribas *et al*., 1994). This could lead to an enhancement of the total peripheral resistance, providing a further mechanism for the hypertension in the transgenic strain (Arribas *et al*., 1994). This notion is in agreement with the observation that treatment which blocks the catecholamine effects, like carvedilol, a nonselective β-blocker with vasodilator properties due to α-adrenoceptor antagonism, is much more effective at reducing blood pressure than hydralazine (Böhm *et al*., unpublished observations). In this context, it is likely that the increased basal sympathetic nervous tone in transgenic rats (Böhm *et al*., 1994) (and possibly further

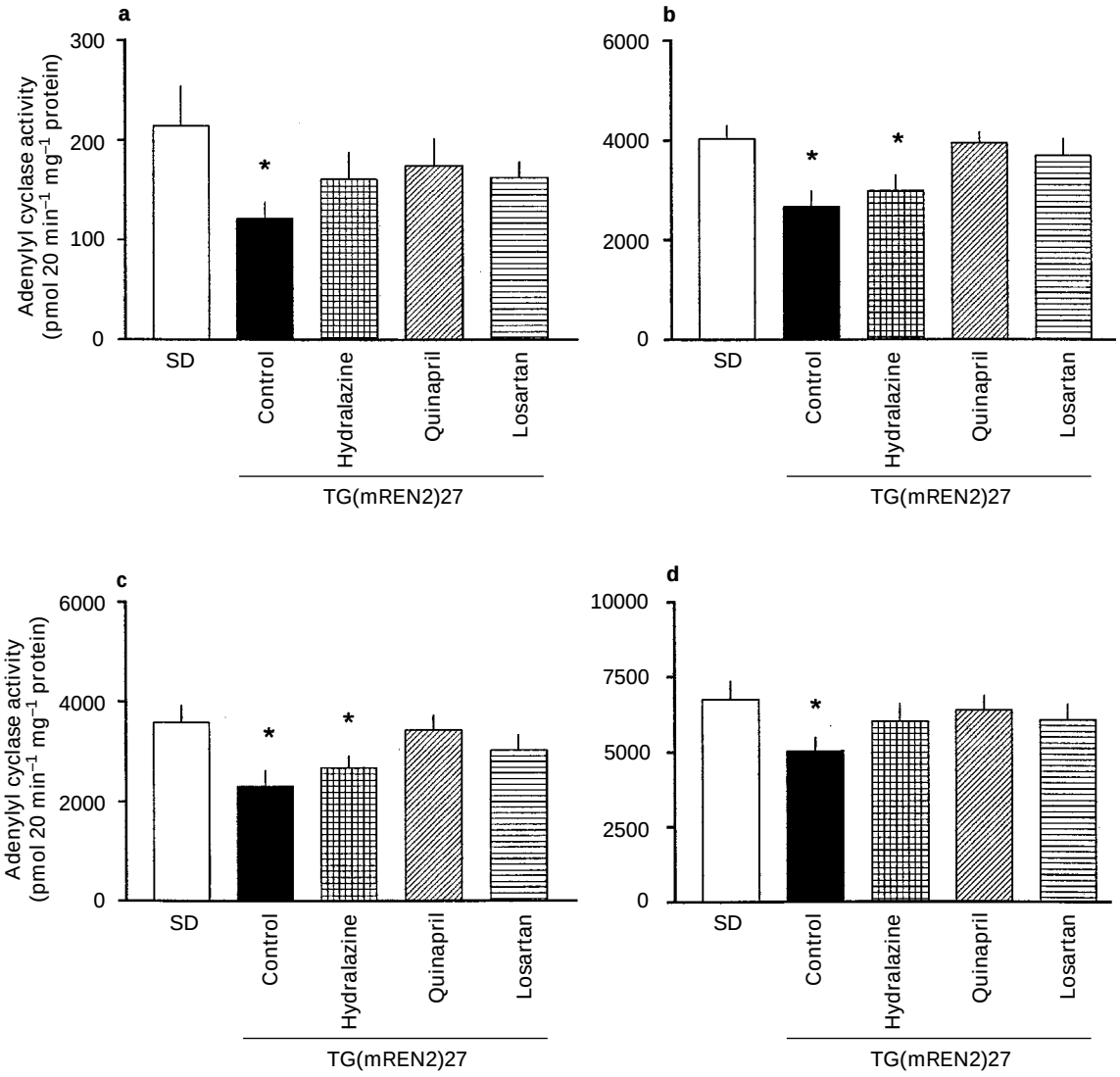

Figure 3 Basal (a), isoprenaline- (b), Gpp(NH)p- (c) and forskolin-stimulated (d) adenylyl cyclase activities in left ventricular membranes from Sprague-Dawley control rats (SD) and transgenic rats (TG(mREN2)27) treated with hydralazine, quinapril, losartan or kept under control conditions. Each column represents mean data ($n=8$) and vertical lines show s.e.mean. *$P<0.05$ vs corresponding values in Sprague-Dawley rats.

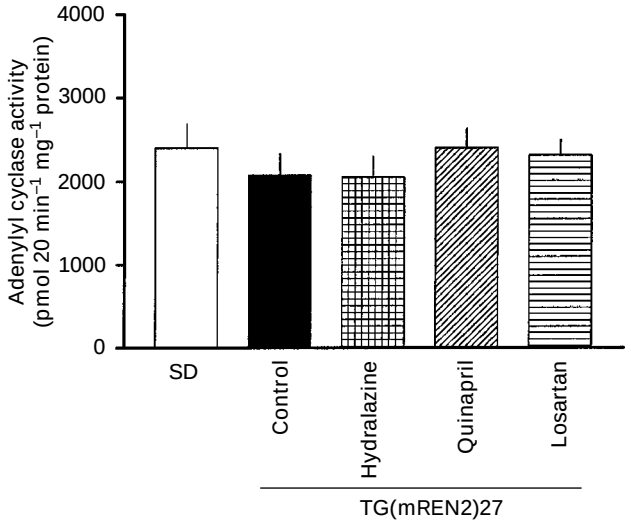

Figure 4 $MnCl_2$ plus forskolin-stimulated adenylyl cyclase activities in left ventricular membranes from Sprague-Dawley control rats (SD) and transgenic rats (TG(mREN2)27) treated with hydralazine, quinapril, losartan or kept under control conditions. Each column represents mean data ($n=8$); vertical lines show s.e.mean.

enhancement of the sympathetic nervous tone as a typical side effect of chronic hydralazine treatment) in concert with RAS-activation may counteract the effects of hydralazine mediated via direct vascular smooth muscle relaxation.

In the development of heart failure, an increase in left ventricular mass and volume, referred to as left ventricular remodelling occurs after pressure overload and has been shown to be an independent risk factor for the development of chronic heart failure (Levy, 1988). At the cellular level, remodelling includes myocyte hypertrophy, myocyte slippage and interstitial fibrosis (Blaufarb & Sonnenblick, 1996). The remodelling stimulus has usually been reviewed as dependent on load and wall stress (Grossman *et al*., 1975). However, recent evidence suggests that hormonal factors may play a crucial role. Increased concentrations of noradrenaline have been shown to promote α-adrenoceptor-mediated growth of isolated cardiomyocytes (Simpson, 1983) and growth promoting effects of angiotensin II on fibroblasts and cardiomyocytes (Sadochima & Izumo, 1993) are well known. Animal studies show regression of cardiac hypertrophy when ACE-inhibitors are administered even at doses that do not lower blood pressure (Linz *et al*., 1989). It is not clear whether this effect is exclusively the result of decreased generation of the growth

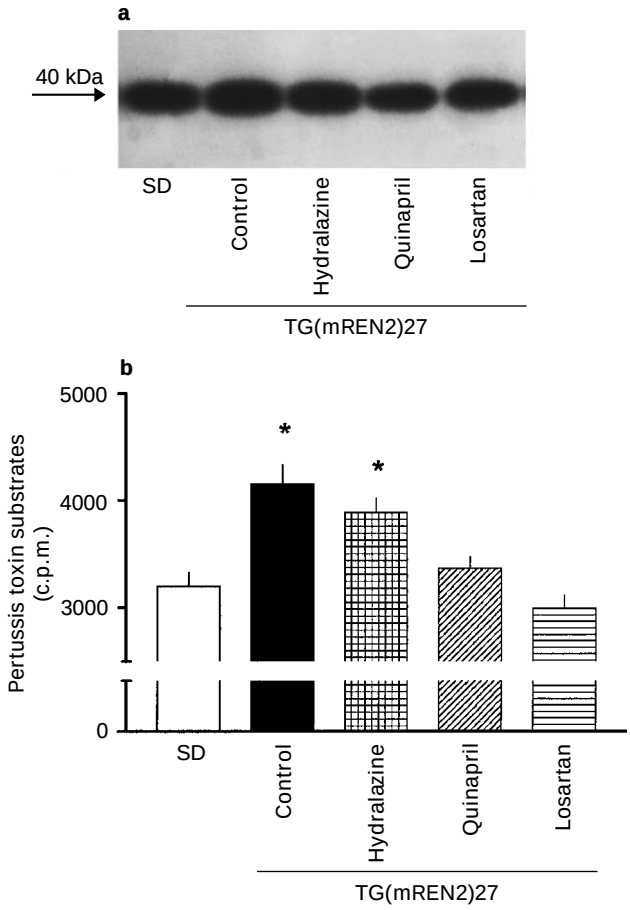

Figure 5 $G_{i\alpha}$-related pertussis toxin substrates (40 kDa) in left ventricular membranes from Sprague-Dawley control rats (SD) and transgenic rats (TG(mREN2)27) treated with hydralazine, quinapril, losartan or kept under control conditions. (a) A representative autoradiogram is shown. (b) Mean values for incorporated [^{32}P]-ADP-ribose as determined by detecting the radioactivity from the 40 kDa-bands. Vertical lines show s.e.mean ($n=8$). *$P<0.05$ vs corresponding values in Sprague-Dawley rats.

promoting hormone angiotensin II. Inhibition of angiotensin converting enzyme is associated with decreased breakdown of bradykinin which may have antiproliferative effects (Weber & Brilla, 1983). The observation that the beneficial effects of ACE-inhibitors can partly be blocked by the bradykinin receptor antagonist Hoe-140 in a dog model of myocardial infarction may confirm this hypothesis (McDonald *et al*., 1995). In the present study, the AT_1-receptor antagonist losartan, which presumably has no influence on bradykinin metabolism, and the ACE-inhibitor quinapril equally normalized left ventricular weights in TG(mREN2)27 rats. Findings from other animal studies are inconsistent. Some studies suggest that ACE-inhibitors and AT_1-antagonists act differentially regarding prevention and regression of left ventricular hypertrophy. These differences were attributed to ACE-independent angiotensin II generation or effects on kinin metabolism (Linz *et al*., 1991; Ruzicka *et al*., 1993) and may depend on the specific animal model investigated. In contrast to quinapril and losartan, hydralazine treatment incompletely reduced left ventricular mass. The small amount of LV hypertrophy regression may be due to the small but significant drop in blood pressure.

Local sympathetic activity was assessed by measurement of neuropeptide Y-concentrations in the left ventricular myocardium. As noradrenaline and neuropeptide Y are exocytotically coreleased from cardiac postganglionic nerve fibres (Haass *et al*., 1989), neuropeptide Y represents a valuable marker of myocardial sympathetic tone. The present and a previous study (Böhm *et al*., 1994) revealed decreased myocardial neuropeptide Y and noradrenaline concentrations in TG(mREN2)27, whereas plasma concentrations of these neurotransmitters were increased. Additionally, heart rates were significantly increased in the transgenic rats as a marker of enhanced sympathetic nervous outflow. Several mechanisms of angiotensin II-dependent facilitation of cardiac sympathetic tone (Mancia *et al*., 1995) may explain this strong activation of the cardiac sympathetic nerves in TG(mREN2)27. Angiotensin II facilitates the exocytotic release of noradrenaline from the sympathetic nerve terminals due to stimulation of presynaptic angiotensin receptors (Malik & Nasjletti, 1976; Majewski *et al*., 1984). Furthermore, increased local sympathetic tone can be produced through a central mechanism via angiotensin II-mediated stimulation of the area postrema.

Quinapril and losartan completely restored the depletion of myocardial neuropeptide Y while hydralazine treatment showed no significant effects. These findings are in good agreement with findings from clinical trials and animal studies. A recent placebo-controlled study showed that noradrenaline plasma concentrations decreased in patients with heart failure who had received the AT_1-receptor antagonist losartan (Crozier *et al*., 1995). In contrast, hydralazine therapy has been shown to be associated with progressive activation of the sympathetic nervous system in patients with severe congestive heart failure (Dalay *et al*., 1986). Captopril-treatment at doses that failed to normalize systolic blood pressure was able to reduce sympathetic activity in spontaneously hypertensive rats (Böhm *et al*., 1995a,b). In the same animal model, a generalized sympathetic hyperactivity was observed during the initial period of hydralazine treatment, whereas a selective increase in ventricular sympathetic nervous activity persisted during long-term hydralazine administration (Tsoporis & Leenen, 1988). The latter finding would parallel the observation from the present study, that heart rates further increased during hydralazine treatment.

Persistent β-agonist stimulation is known to cause desensitization of the β-adrenoceptor-G-protein-adenylyl cyclase complex (Hausdorff *et al*., 1990; Hadcock & Malbon, 1993). In TG(mREN2)27-rats, the increased sympathetic nervous outflow in the left ventricular myocardium was associated with a downregulation of the β-adrenoceptor number and a significant decrease in isoprenaline- and Gpp(NH)p-stimulated adenylyl cyclase activity. This alteration was due to increased activity of inhibitory G-proteins. Furthermore, adenylyl cyclase stimulation by forskolin, which is known to activate the catalytic subunit of adenylyl cyclase directly (Seamon *et al*., 1981), was significantly depressed in left ventricular myocardium from TG(mREN2)27-rats compared to controls. Nevertheless, forskolin-stimulated enzyme activity is modulated by G-proteins (Seamon *et al*., 1981; Darfler *et al*., 1982; Taussig *et al*., 1993). Since catalyst activity of adenylyl cyclase, as assessed by forskolin plus Mn^{2+}-co-stimulation, did not differ significantly between Tg(mREN2) 27-rats and SP-controls, desensitization of adenylyl cyclase to forskolin-stimulation is suggested to be due to the upregulation of $G_{i\alpha}$-activity. The alterations of the sympathetic neuroeffector mechanism are associated with an attenuated inotropic response of the myocardium to β-agonist stimulation (Böhm *et al*., 1995). Quinapril and losartan similarly prevented the downregulation of β-adrenoceptors, increases in $G_{i\alpha}$ activities and decreases in adenylyl cyclase activities in TG(mREN2)27. Parallel to the still elevated (compared to

SD) and treatment insensitive (compared to TG(mREN2)27) sympathetic tone in the hydralazine-treated group, $G_{i\alpha}$ activity, β-adrenoceptor densities and Gpp(NH)p- as well as isoprenaline-stimulated adenylyl cyclase activities did not significantly change compared to untreated TG(mREN2)27.

Because of the lack of vital human tissue available from hypertensive patients, experiments on alterations of the sympathetic neuroeffector mechanisms have been performed in several animal models of genetic or acquired hypertension. Most of these models exhibit similar alterations of the β-adrenoceptor-adenylyl cyclase system as those observed in the TG(mREN2)27 strain (Castellano & Böhm, 1997). It is interesting to note that an increase of inhibitory G-proteins also occurs early during the development of hypertensive heart disease in man (Böhm *et al*., 1995a,b).

In conclusion, the effects of chronic administration of the AT_1-antagonist losartan on neuroeffector defects and cardiac hypertrophy in TG(mREN2)27 are comparable to the effects of the ACE-inhibitor quinapril. However, it is important to note that the haemodynamic and biochemical parameters determined in the present study represent the net effect of possibly very complex physiological responses to the pharmacological treatment. Schunkert *et al*. (1993) provide data that demonstrate a negative feed back regulation of ACE-activity and mRNA-levels by angiotensin II. It is intriguing to speculate that chronic administration of ACE-inhibitors may induce ACE-synthesis, thereby attenuating the tissue drug effect. Some of the beneficial effects of ACE-inhibitors have been attributed to the accumulation of the vasoactive peptide bradykinin and increased production of prostaglandins following kininase II-inhibition (Swartz *et al*., 1980; Cachefeiro *et al*., 1992). However, alternative formation of angiotensin II by cardiac enzymes like chymase (Urata *et al*., 1995) which are not blocked by ACE-inhibitors, may compromise the full effects of these agents. Ruzicka *et al*. (1993) speculated that increased angiotensin II generation, despite ACE-inhibition, may account for the differential effects of ACE-inhibition and the AT_1-receptor blockade on ventricular hypertrophy in a rat-model of volume overload-induced cardiac hypertrophy, while both treatment regimens had similar effects on haemodynamics. Nevertheless, the observation that local angiotensin II formation was completely blocked by ACE-inhibition, in isolated perfused hearts of the rat (Lindpaintner *et al*., 1990), suggest that ACE is the major angiotensin II-forming enzyme. Although the occurrence of chymase-like enzymes has not been shown in the rat heart, this mechanism could be relevant in man (Urata *et al*., 1995). The finding that hydralazine treatment is inferior to RAS-inhibition supports the proposal, that vasodilators with antagonistic effects on neuroendocrine activation are more efficient at preventing structural and cellular alterations of the myocardium. Inhibition of neuroendocrine systems may be a promising approach to delay or prevent the progression from asymptomatic hypertensive cardiomyopathy to heart failure.

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