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Contribution of the renin–angiotensin system to subsensitivity of soluble guanylyl cyclase in TGR(mREN2)27 rats

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Abstract

Soluble guanylyl cyclase activity and its stimulation by diethylamineNONOate was measured in aortae from hypertensive TGR(mREN2)27 rats (TGR) and Sprague–Dawley controls. Superoxide dismutase was added in vitro to evaluate the contribution of oxidative breakdown of nitric oxide (NO) by superoxide anions. Expression of soluble guanylyl cyclase was assessed by reverse transcriptase-polymerase chain reaction (RT-PCR). Basal and stimulated soluble guanylyl cyclase activity was significantly reduced in TGR rats, addition of superoxide dismutase had no effect. Expression of soluble guanylyl cyclase subunits was not different between strains. The independent contribution of hypertension and the overactive renin–angiotensin system to soluble guanylyl cyclase subsensitivity was assessed after normalization of TGR's blood pressure by the Ca^{2+}-channel blocker amlodipine or the angiotensin converting enzyme-inhibitor enalapril. Soluble guanylyl cyclase activity in TGR was slightly increased by amlodipine and almost completely restored by enalapril. In conclusion, TGR showed desensitized vascular soluble guanylyl cyclase, depending on their overactive renin–angiotensin system.

Keywords: Guanylyl cyclase, soluble; Superoxide anion; Aorta; Renin–angiotensin system; Hypertension

1. Introduction

Transgenic hypertensive TGR(mREN2)27 rats (TGR) represent a genetic model of hypertension. They are characterized by fulminant hypertension, an overactive tissue renin–angiotensin system (Véniant et al., 1995) and an inverse circadian blood pressure pattern (Lemmer et al., 1993). Secondary complications such as nephrosclerosis (Bachmann et al., 1992), myocardial hypertrophy (Villarreal et al., 1995) and vascular damage (Struijker-Boudier et al., 1996) appear at an early age. In vitro studies have demonstrated reduced endothelium-dependent relaxation in aortic ring preparations from male TGR (Pinto et al., 1997) similar to findings in other models of experimental hypertension (Dominiczak and Bohr, 1995). Impaired vascular reactivity could depend on an oxidative breakdown of nitric oxide (NO), due to enhanced formation of superoxide anions (O_2^-) as observed in rats with experimental heart failure (Bauersachs et al., 1999) and in hypercholesterolemic rabbits (Minor et al., 1990). Alternatively, desensitization of the soluble guanylyl cyclase could play a role which upon stimulation by NO catalyzes the conversion of GTP into the vasodilating second messenger cGMP in vascular smooth muscle. We have recently shown that soluble guanylyl cyclase activity in vitro is reduced in TGR aorta under basal conditions and after stimulation by the NO-donor sodium nitroprusside (Witte et al., 1998). Investigations with purified soluble guanylyl cyclase showed that enzymatic activity of this heterodimer is stimulated 100- to 200-fold by NO (Stone and Marletta, 1994); but in our previous experiments, cyanide containing sodium nitroprusside increased cGMP formation no more than about 70-fold. We suspected that sodium nitroprusside-stimulated soluble guanylyl cyclase activity could have been influenced by the release of five cyanide anions with each molecule of NO. Additionally, sodium nitroprusside does not spontaneously release NO, but requires the presence of sulfhydryl donors. To exclude a possible influence of cyanide on soluble guanylyl cyclase activity, the NO-donor diethylamineNONOate was used in this study of

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Sprague–Dawley and TGR rats. This compound does not contain cyanide, generates NO spontaneously in a first order kinetic rate and does not require thiol groups for NO release (Morley and Keefer, 1993).

Several studies have indicated that enhanced formation of O_2^- leads to an increased oxidative breakdown of NO in vivo (McIntyre et al., 1999). Therefore, we investigated whether O_2^- could reduce the amount of NO available under in vitro conditions and, thus, explain the decreased NO-stimulated soluble guanylyl cyclase activity in TGR aorta. To exclude an influence of O_2^- on soluble guanylyl cyclase activity, measurements of enzyme activity was carried out in the presence and absence of superoxide dismutase. An additional mechanism leading to depressed soluble guanylyl cyclase activity has recently been shown by Gupta et al. (1997): the authors transfected the β_2-subunit in rat aortic smooth muscle cells, thereby demonstrating inhibition of stimulation of the α_1/β_1 form of soluble guanylyl cyclase by NO. Moreover, in renal tissue from Dahl salt-sensitive rats, they demonstrated an increase in the endogenous α_1/β_2 heterodimer and a decrease in the α_1/β_1 (Gupta et al., 1997), which could explain the decreased renal guanylyl cyclase activity observed in the salt-sensitive rats (Simchon et al., 1996). It is known that the additional mouse renin gene in TGR affects not only the activity of the renin–angiotensin system, but also influences the expression of several other proteins, e.g. enzymes involved in adrenal steroid biosynthesis (Sander et al., 1994), and a number of cardiac proteins such as actin, laminin and collagen (Ohta et al., 1996). Based on these findings, we suspected that changes in the subunit composition of soluble guanylyl cyclase might be present in TGR vasculature and, thereby, influence the responsiveness of the enzyme to NO. Therefore, we studied the subunit composition of soluble guanylyl cyclase in aortic tissue from Sprague–Dawley and TGR rats using reverse transcriptase-polymerase chain reaction (RT-PCR).

In a second series, we examined whether the reduced soluble guanylyl cyclase activity in TGR depends on hypertension per se or on the overactive renin–angiotensin system in the transgenic animals. This issue was addressed by prolonged treatment of TGR with antihypertensive drugs of two different classes: the Ca^{2+}-channel blocker amlodipine and the angiotensin converting enzyme-inhibitor enalapril. In order to differentiate between the influence of blood pressure and that of the renin–angiotensin system, dosages of both drugs were titrated until comparable reductions in blood pressure were achieved.

2. Methods

2.1. Animals

Six-week-old male Sprague–Dawley ($n = 19$) and age-matched transgenic TGR rats ($n = 43$) were obtained from M&B (Ry, Denmark). Animals were kept singly in plastic cages under a 12:12 h light/dark regimen with lights on at 0700 h, under constant environmental conditions. They had free access to food and water.

Untreated TGR and Sprague–Dawley rats were used to investigate the involvement of O_2^- in NO-sensitive soluble guanylyl cyclase activity ($n = 5$ per strain), and for studies on soluble guanylyl cyclase subunit expression ($n = 6$ per strain). At the age of 12 weeks, the animals were killed by decapitation in enflurane anesthesia. The thoracic aorta was dissected, freed from connective tissue, rinsed in ice-cold isotonic saline solution, dried on filter paper, frozen in liquid nitrogen and stored at -60°C.

Radiotransmitters for continuous monitoring of blood pressure, heart rate and locomotor activity (TA11PA-C40, Data Sciences International, St. Paul, MN, USA) were implanted into 24 TGR, eight animals per group. Additional TGR rats ($n = 8$) without transmitter were assigned to the untreated control group to cover the expected loss of animals due to the spontaneous mortality. Surgery was done in general anesthesia using enflurane (Abbott, Wiesbaden, Germany) as described in detail (Lemmer et al., 1993). All experiments were performed in adherence to the Guide for the Care and Use of Laboratory Animals as published by the National Institutes of Health, and were approved by German federal regulations (RP Karlsruhe, Az. 35-9185.81/1/99).

2.2. Treatment study protocol

At the age of 12 weeks, 4 weeks after transmitter implantation, TGR received the Ca^{2+}-channel blocker amlodipine (amlodipine group, $n = 8$) or the angiotensin converting enzyme-inhibitor enalapril (enalapril group, $n = 8$) as an addition to the animals' drinking water. The drug intake was monitored throughout the study by weighing the water bottles. In the first 2 weeks of treatment, the drug dosage was adjusted until a 24 h-blood pressure of about 140/90 mm Hg was achieved in both treatment groups. Baseline values were taken from the last week prior to drug dosing. The treatment values were calculated as the average of the whole treatment period excluding the 2 weeks of the dose-titration phase. After a treatment period of 8 weeks TGR and untreated, age-matched Sprague–Dawley controls were sacrificed and the thoracic aorta was prepared as described above.

2.3. Measurement of soluble guanylyl cyclase activity

2.3.1. Tissue preparation

Each thoracic aorta was homogenized in 2 ml ice-cold assay buffer (50 mM Tris–HCl, 5 mM $MgCl_2$, 1 mM DL-dithiothreitol, pH 7.4) with a Potter S glass homogenizer (Braun, Melsungen, Germany). DL-Dithiothreitol was used to protect soluble guanylyl cyclase against oxidation. The suspension was centrifuged for 10 min (4°C) at 25 000

$\times g$. The resulting supernatant was used for measurement of soluble guanylyl cyclase activity. All preparation steps were performed at 4°C to prevent thermal alteration of the enzyme.

2.3.2. Guanylyl cyclase assay

Basal and stimulated soluble guanylyl cyclase activity was measured as described by Witte et al. (1995). Briefly, 100 μl supernatant of the tissue preparation was added to prewarmed assay buffer containing 0.5 mM GTP, 10 mM phosphocreatine, 0.1 g/l creatine phosphokinase (Roche, Mannheim, Germany), 1 mM 3-isobutyl-1-methylxanthine (Sigma, Deisenhofen, Germany). Stimulation of soluble guanylyl cyclase was performed by addition of the NO-donor diethylamineNONOate (Calbiochem, Bad Soden, Germany) in a concentration range of 0.3–30 μM. In the first experimental series, the incubation was carried out with and without addition of superoxide dismutase (Sigma) 300 units/ml . After 6 min incubation at 37°C the enzyme reaction was stopped by heating at 120°C and samples were centrifuged at $10\,000 \times g$. The [^{3}H] cGMP assay (TRK 500, Amersham Life Science, Germany) was used to determine the amount of cGMP formed.

2.4. RT-PCR analysis of soluble guanylyl cyclase subunits

2.4.1. RNA preparation

Total RNA of thoracic aorta from Sprague–Dawley and TGR rats was prepared as described previously (Behrends et al., 2000), and the concentration was determined photometrically at 260 nm.

2.4.2. First strand cDNA synthesis

First strand synthesis was performed using a kit with moloney murine leukemia virus reverse transcriptase (Stratagene, Amsterdam, Netherlands) as described previously (Behrends et al., 2000). The equivalent of 100 ng reverse transcribed total RNA was used in the subsequent PCR reactions.

2.4.3. Primers and conditions for PCR analysis

Amplification with AmpliTaq Gold (Perkin Elmer, Norwalk, USA) was performed in a total volume of 50 μl with 40 μl mineral oil overlay in a following final concentration: primers 1 μM each, dNTPs 200 μM, $MgCl_2$ 1.5 mM, AmpliTaq Gold 0.025 U/μl. The following temperature profile was run on a MWG Biotech Thermocycler: samples were denaturated and the polymerase activated by an initial 95°C for 10 min. Thirty-five cycles of denaturation (94°C, 1 min) annealing (50–55°C, 1 min) and extension (72°C, 1 min) were followed by a final extension step at 72°C for 10 min. The further conditions for the primer pairs were:

β_1-subunit: β_1 rat 1069 TCC CTC TCC ATG ATG CTA CAC G and β_1 rat1585 TCG CCA TCT ACT TGA ACT TGA CCA; 517 bp PCR-product, annealing temperature 55°C;

β_2-subunit: primer b2hum543 AGG GAA GAA GGA GCA TGT TGT GTT and mmb2_435 TGA GGG TGG ACG ATG GAG AAA TAC; 429 bp PCR-product, annealing temperature 55°C;

α_1-subunit: primer α_1 _146 TGY TAY GAR GAR GAY GAR TAY AT and α_1 _340 AAY TGC ATR TTI ARC ATI GTC AT, 608 bp PCR-product, annealing temperature 55°C;

α_2-subunit: primer a2svsense TGT ACA CCA GAT TTG ACC ACC AGT and a2svsanti ACG AGA CCG CGG AAT GAA TG; 392 bp PCR-product, annealing temperature 55°C.

Amplified PCR products were separated on 1% (w/v) agarose gels and stained with ethidium bromide.

2.5. Protein concentration

Protein content in the supernatant was measured by using the Coomassie® Plus assay (Pierce, oud-Beijerland). Bovine serum albumin (Sigma) was dissolved in assay buffer and taken as standard.

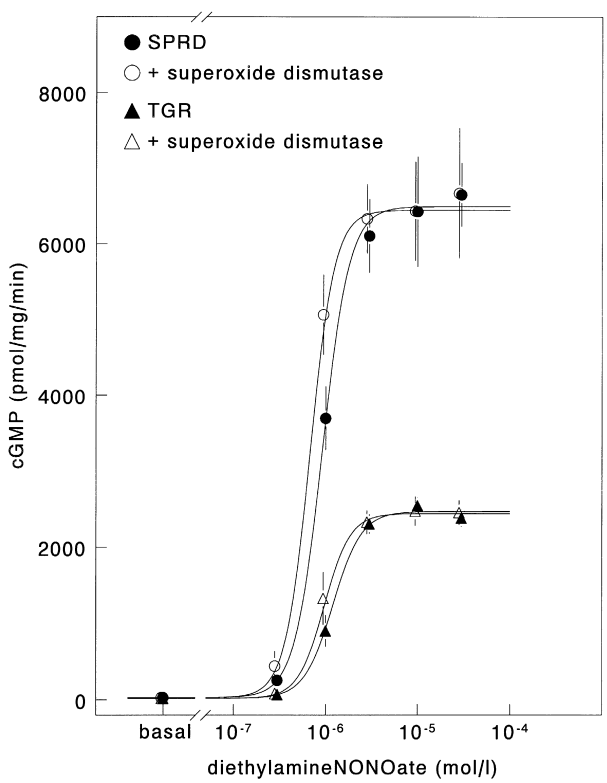

Fig. 1. Formation of cGMP under basal conditions and after NO-stimulation by the NO-donor diethylamineNONOate in aortic tissue from normotensive 12-week-old Sprague–Dawley (SPRD) and transgenic hypertensive TGR rats. The maximum increase in cGMP formation (E_{max}) by diethylamineNONOate was lower in TGR than in Sprague–Dawley rats, while the affinity (pD_2) was unchanged. Addition of superoxide dismutase (300 U/ml) could not enhance activity of soluble guanylyl cyclase in TGR aorta. Means ± S.E.M., $n = 5$ per strain.

Table 1
Aortic soluble guanylyl cyclase in 12-week-old Sprague–Dawley and hypertensive TGR rats: enzyme activity in the absence and presence of superoxide dismutase

Strain	In vitro addition	Basal cGMP formation (pmol mg^{-1} min^{-1})	Stimulation by diethylamineNONOate	
			pD_2 ($-\log$ mol/l)	E_{max} (pmol mg^{-1} min^{-1})
Sprague–Dawley ($n = 5$)	none	30.5 ± 4.0	6.04 ± 0.11	6309 ± 1285
	superoxide dismutase	30.5 ± 4.2	6.13 ± 0.11	6473 ± 948
TGR ($n = 5$)	none	19.6 ± 4.1	5.89 ± 0.16	2547 ± 175
	superoxide dismutase	20.4 ± 5.4	6.00 ± 0.19	2570 ± 228
ANOVA	strain	$P < 0.05$	n.s.	$P < 0.0001$
	superoxide dismutase	n.s.	n.s.	n.s.
	interaction	n.s.	n.s.	n.s.

Mean values ± S.D., E_{max} = maximum increase in cGMP formation, pD_2 = negative decadic logarithm of EC_{50}, bivariate ANOVA.

2.6. Statistics

Concentration–response curves to diethylamineNONOate were analyzed by using Pharmfit (Mattes et al., 1991), and the pD_2 (negative decadic logarithm of the EC_{50}) and E_{max}-values (maximum increase in cGMP formation) were obtained from the fitted curves. Bivariate analysis of variance (ANOVA) was used to test for influ-

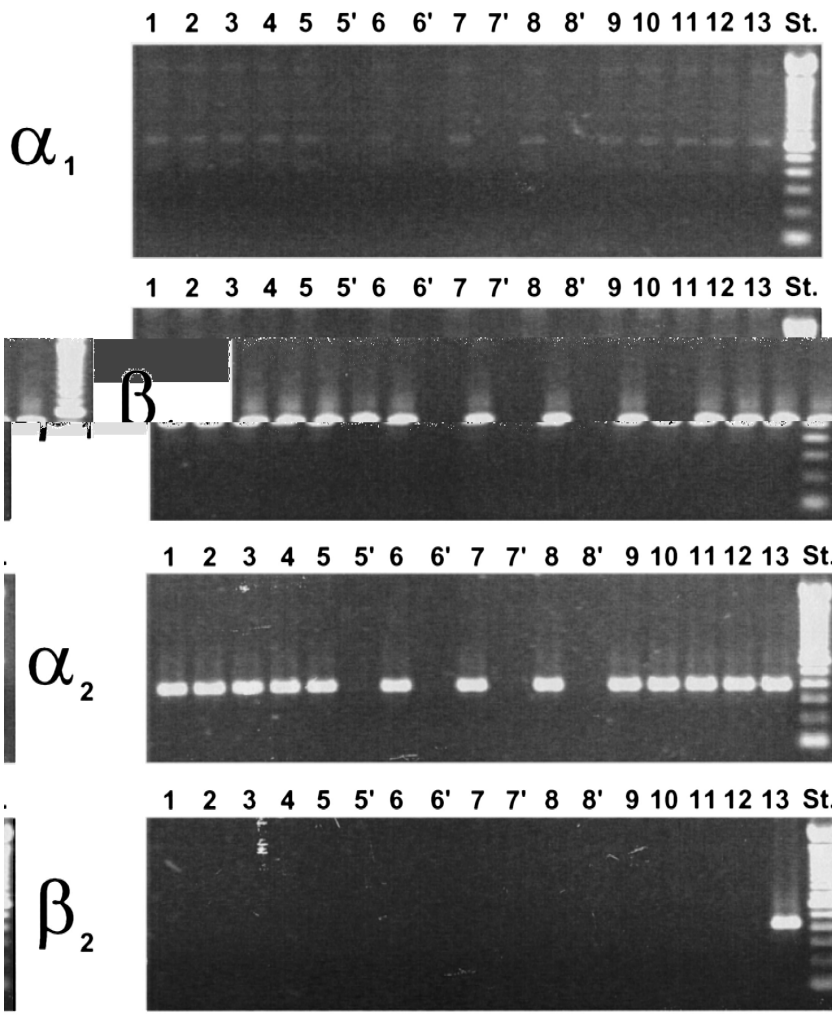

Fig. 2. RT-PCR analysis of soluble guanylyl cyclase subunit distribution in aortic tissue from Sprague–Dawley (odd numbers) and hypertensive TGR rats (even numbers). Amplified PCR products were separated on 1% agarose gels and stained with ethidium bromide. The subunit distribution was not different between the strains. Numbers with apostrophy (5′, 6′, 7′, 8′) represent negative controls of both strains, with the addition of water instead of reverse transcriptase. 13 = rat kidney, St. = standard.

ence of strain and age, and for the effects of superoxide dismutase on enzyme activity in both strains. Differences between treatment groups were tested by univariate ANOVA and by the non-parametric Kruskal–Wallis test, where appropriate. The statistics software BiAS 6.0 (Ackermann, 1997) was used.

3. Results

3.1. Aortic soluble guanylyl cyclase activity in 12-week-old Sprague–Dawley and TGR rats

Basal soluble guanylyl cyclase activity and the maximum increase in cGMP formation (E_{max}) by diethylamineNONOate were significantly lower in TGR aortae than in Sprague–Dawley controls, while the affinity (pD_2) was unchanged (Fig. 1). Furthermore, the relative increase in aortic soluble guanylyl cyclase activity by diethylamineNONOate (E_{max}/basal cGMP formation) was significantly reduced in the transgenic strain (131.6 ± 32.2-fold basal) compared with Sprague–Dawley controls (205.2 ± 27.0-fold basal).

The addition of superoxide dismutase 300 units/ml did not change basal and NO-stimulated soluble guanylyl cyclase activity (ANOVA, n.s.), neither in Sprague–Dawley nor in TGR rats (Table 1).

3.2. RT-PCR analysis of soluble guanylyl cyclase subunits in Sprague–Dawley and TGR rats

Expression of soluble guanylyl cyclase subunit mRNA in aortic tissue from TGR rats did not differ from Sprague–Dawley controls (Fig. 2). The predominant subunits in Sprague–Dawley as well as in TGR aortae were α_2 and β_1. Aortic tissue from both strains showed low expression of α_1-subunits, and definitely no mRNA expression of the β_2-subunit.

3.3. Treatment study

3.3.1. Effects of treatment on cardiovascular parameters

Six TGR died during the study, four untreated TGR, one of the amlodipine and one of the enalapril group. One additional untreated TGR developed symptoms of malignant hypertension and excessively high blood pressure, and required intermittent captopril treatment. This animal was excluded from all further analyses.

To achieve a 24 h blood pressure of about 140/90 mm Hg rats in the amlodipine group received 38.9 ± 11.9 mg/kg per day amlodipine and in the enalapril group 3.5 ± 0.8 mg/kg per day enalapril.

At baseline the 24 h means of diastolic and systolic blood pressure in the amlodipine, enalapril and untreated TGR group did not differ from each other (Fig. 3). During

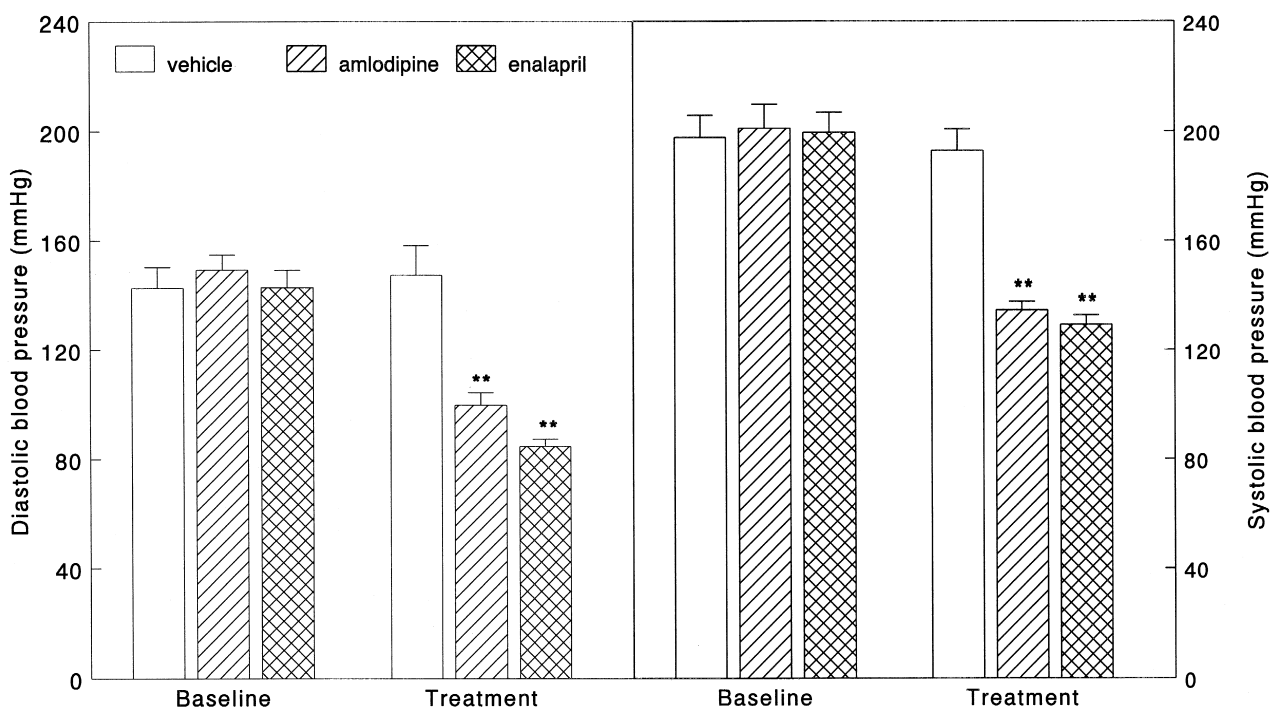

Fig. 3. Diastolic and systolic blood pressure at baseline and during the treatment period in untreated (vehicle), amlodipine and enalapril-treated transgenic hypertensive TGR rats. At baseline, the 24 h means in diastolic and systolic blood pressure did not differ between the groups. During the treatment period blood pressure was significantly lower in the amlodipine and enalapril group than in untreated TGR. Means ± S.E.M., $n = 7$ per strain, $^{**}P < 0.001$, analysis of variance followed by Scheffé's test for pairwise comparisons.

the treatment period diastolic and systolic blood pressure were significantly lower in the amlodipine and enalapril groups than in untreated TGR. Additionally, diastolic blood pressure was slightly more decreased by enalapril than by amlodipine, while amlodipine and enalapril lowered systolic blood pressure to similar values. Blood pressure in untreated TGR peaked at 12 weeks of age (diastolic blood pressure 155 ± 28 mm Hg, systolic blood pressure 206 ± 25 mm Hg) and declined thereafter. At 20 weeks of age, 24 h means in diastolic and systolic blood pressure were 134 ± 27 and 178 ± 24 mm Hg, respectively.

3.3.2. Effects of treatment on aortic soluble guanylyl cyclase activity

Basal soluble guanylyl cyclase activity in thoracic aorta showed no significant differences between Sprague–Dawley, untreated, amlodipine and enalapril-treated TGR rats (Table 2). NO-stimulated soluble guanylyl cyclase activity was significantly lower in untreated TGR than in untreated Sprague–Dawley controls (Fig. 4), and the maximum increase in cGMP formation (E_{max}) by diethylamineNONOate was blunted in the transgenic animals.

Treatment with amlodipine and enalapril resulted in an increased NO-stimulated soluble guanylyl cyclase activity, which was no longer different from normotensive Sprague–Dawley controls (Table 2). After 8 weeks treatment with enalapril improvement in NO-stimulated soluble guanylyl cyclase activity was greater than after treatment with amlodipine. In the presence of 30 μM diethylamineNONOate, cGMP formation was significantly increased in the enalapril group compared to untreated TGR (Fig. 4). The potency (pD_2) of diethylamineNONOate did not differ between the four groups.

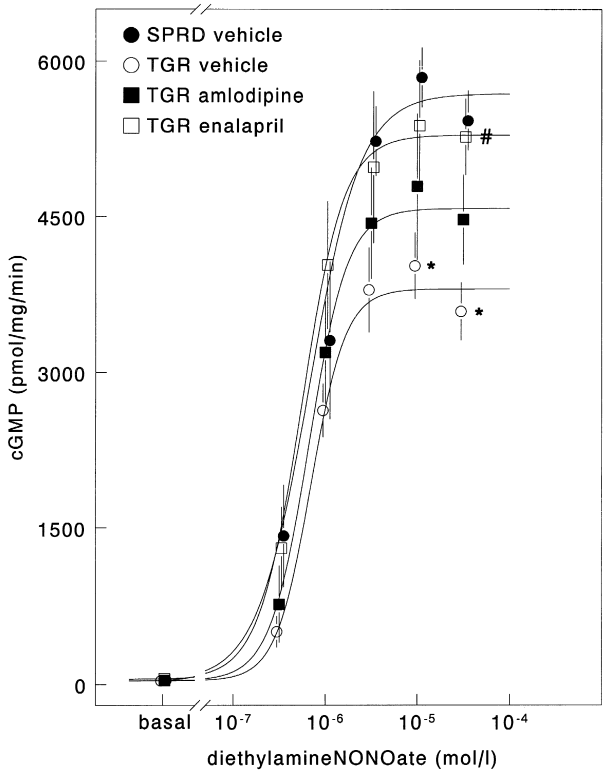

Fig. 4. Formation of cGMP under basal conditions and after NO-stimulation by the NO-donor diethylamineNONOate in aortic tissue from 20-week-old normotensive Sprague–Dawley rats (SPRD), untreated (vehicle), amlodipine and enalapril-treated transgenic hypertensive TGR rats. The NO-stimulated soluble guanylyl cyclase activity was significantly lower in untreated TGR than in Sprague–Dawley controls. Eight weeks of antihypertensive treatment led to an increase in NO-stimulated soluble guanylyl cyclase activity, which was greater in the enalapril than in the amlodipine group. Means ± S.E.M., $n = 5–7$ per strain, $^{*}P < 0.05$ vs. Sprague–Dawley, $^{\#}P < 0.05$ vs. untreated TGR, non-parametric Kruskal–Wallis test followed by Dunn's pairwise comparisons.

3.4. Age-dependent changes in aortic soluble guanylyl cyclase activity

In both strains, basal enzyme activity was higher than in 12-week-old animals of the first study. Bivariate ANOVA revealed a significant influence of strain ($P < 0.05$) and age ($P < 0.01$), and there was no significant interaction between both parameters. The NO-stimulated cGMP formation (E_{max}) was consistently lower in the TGR strain (bivariate ANOVA, strain $P < 0.0001$). However, in 20-week-old Sprague–Dawley rats, E_{max} was lower than in 12-week-old animals whereas, in TGR, E_{max} was increased with aging. Therefore, bivariate ANOVA failed to detect a significant influence of age, due to the interaction between age and strain ($P < 0.05$). The potency (pD_2) of

Table 2
Aortic soluble guanylyl cyclase in 20-week-old Sprague–Dawley and hypertensive TGR rats: influence of antihypertensive treatment in TGR rats

Strain	Treatment	Basal cGMP formation (pmol mg^{-1} min^{-1})	Stimulation by diethylamineNONOate	
			pD_2 (−log mol/l)	E_{max} (pmol mg^{-1} min^{-1})
Sprague–Dawley ($n = 6$)	vehicle	39.5 ± 6.7	6.17 ± 0.35	5744 ± 667
TGR ($n = 7$)	vehicle	34.5 ± 13.8	6.14 ± 0.12	3791 ± 796[a]
TGR ($n = 5$)	amlodipine	36.1 ± 15.2	6.19 ± 0.22	4563 ± 1221
TGR ($n = 6$)	enalapril	54.0 ± 36.2	6.20 ± 0.17	5202 ± 1256
ANOVA		n.s.	n.s.	$P < 0.05$

Mean values ± S.D., E_{max} = maximum increase in cGMP formation, pD_2 = negative decadic logarithm of EC_{50}, univariate ANOVA followed by Scheffé's test for pairwise comparisons, n.s. = not significant.

[a] $P < 0.05$ TGR vs. Sprague–Dawley.

diethylamineNONOate was not influenced by age and strain.

4. Discussion

The present study demonstrates that specific soluble guanylyl cyclase activity and its stimulation by NO is reduced in thoracic aorta from 12-week-old hypertensive TGR compared to age-matched Sprague–Dawley controls. Superoxide dismutase had no influence on basal and NO-stimulated soluble guanylyl cyclase activity in Sprague–Dawley and TGR rats. These findings suggest that the depressed NO-sensitive soluble guanylyl cyclase activity in TGR is not due to a reduced amount of NO available in vitro, but reflects a disturbed function of the soluble guanylyl cyclase itself. This assumption is in agreement with a study in spontaneously hypertensive rats (SHR), which also showed a decreased NO-dependent activation in aortic tissue (Klöß et al., 2000).

In 20-week-old untreated TGR of the second study, basal soluble guanylyl cyclase activity was not different from age-matched Sprague–Dawley rats. This discrepancy between 12- and 20-week-old rats cannot easily be explained. However, taking into account that 4 out of 16 untreated TGR died between 12 and 20 weeks of age, the surviving rats may represent a selected population of less severely diseased animals. Furthermore, in agreement with previously published data by Lee et al. (1996), we observed that blood pressure decreased with aging in the untreated TGR group, suggesting that changes in blood pressure per se could have an impact on the activity of vascular soluble guanylyl cyclase. However, the age-dependent decrease in NO-stimulated cGMP formation in Sprague–Dawley aorta is unlikely to reflect changes in blood pressure, because blood pressure in normotensive Sprague–Dawley rats does not change from 12 to 20 weeks of age (Witte et al., 1999). Therefore, other mechanisms must be involved such as a reduced expression of the β-subunit of soluble guanylyl cyclase with aging, which has been shown in normotensive (Chen et al., 2000) and SHR (Klöß et al., 2000). Unfortunately, we are not able to conclusively answer this question, because our study did not specifically address the issue of age-related changes, but was mainly focussed on the contribution of blood pressure and the renin–angiotensin system to regulation of soluble guanylyl cyclase activity.

The results of our treatment study suggest that hypertensive blood pressure per se could have an influence on vascular soluble guanylyl cyclase, because normalisation of blood pressure in TGR by chronic treatment with amlodipine and enalapril led to an improvement of NO-sensitive soluble guanylyl cyclase activity. However, the impaired NO-sensitivity of soluble guanylyl cyclase in TGR was not a simple consequence of hypertension alone, because treatment with amlodipine, which does not inhibit the renin–angiotensin system, did not increase soluble guanylyl cyclase activity to the same extent as enalapril. The greater effectiveness of the angiotensin converting enzyme-inhibitor suggests that the overactive renin–angiotensin system in TGR contribute to the impairment of soluble guanylyl cyclase activity.

Different molecular mechanisms may be involved in the disturbed NO-sensitivity of soluble guanylyl cyclase by hypertension and an increased angiotensin II formation.

It is known that increased shear stress in hypertension evokes a higher Ca^{2+} influx in endothelial cells, followed by activation of endothelial NO synthase (eNOS) (Busse and Fleming, 1998). The resulting formation of NO counterregulates high blood pressure by relaxation of vascular smooth muscle. NO activates soluble guanylyl cyclase by forming a nitrosyl–heme complex with the heme iron. However, under increased NO concentrations NO desensitizes the vascular soluble guanylyl cyclase by partially oxidizing its heme iron, thereby preventing the formation of the nitrosyl–heme complex and, thus, the activation of soluble guanylyl cyclase (Brandes et al., 2000). In addition, the increased cGMP downregulates NO formation by eNOS. Earlier studies demonstrated that cGMP is involved as a mediator in negative-feedback regulation of the expression of eNOS (Vaziri and Wang, 1999) and soluble guanylyl cyclase (Filippov et al., 1997).

Additionally, Gupta et al. (1997) demonstrated that transfection of the β_2-subunit in aortic smooth muscle cells led to inhibition of the stimulation of the α_1/β_1 form of soluble guanylyl cyclase. Since no data were available on the endogenous expression of the β_2-subunit in rat aorta, we have studied soluble guanylyl cyclase subunit expression by RT-PCR. Our findings show that the β_2-subunit is not expressed in aortic tissue, neither in Sprague–Dawley nor in TGR rats. Obviously, the observed soluble guanylyl cyclase subsensitivity in TGR must depend on other mechanisms.

Besides hypertension, an overactive renin–angiotensin system is characteristic for TGR, and angiotensin II is known to activate eNOS via angiotensin AT_1 receptors (Saito et al., 1996). Under physiological conditions, the increase of eNOS activity is a useful mechanism to prevent a too pronounced increase in blood pressure by angiotensin II. However, in the presence of an overactive renin–angiotensin system, as observed in TGR, chronically elevated angiotensin II levels may result in constantly activated eNOS which could lead to desenzititation of the NO-dependent soluble guanylyl cyclase as described above. In support of this hypothesis, experiments with eNOS knockout mice showed that the removal of endogeneous NO leads to supersensitivity of vascular soluble guanylyl cyclase (Brandes et al., 2000).

Kojda et al. (1998) could demonstrate that excess O_2^- production in aortic tissue from SHR may cause a desensitization of vascular soluble guanylyl cyclase. Therefore, it is possible that subsensitivity of soluble guanylyl cyclase

in TGR rats could also depend on an excess O_2^- production in vivo. Angiotensin II is known to stimulate the vascular NADPH oxidase (Griendling et al., 1994) leading to formation of a great amount of O_2^-. Superoxide anions could play a dual role: they reduce amount of the NO available and, additionally, could desensitize soluble guanylyl cyclase by oxidizing its heme iron. It is likely that TGR rats are characterized by an increased O_2^- production in aortic tissue, similar to findings in hypertensive SHR (Kojda et al., 1998), and in rats receiving an angiotensin II infusion for 3 days (Wattanapitayakul et al., 2000).

In conclusion, our present findings demonstrate a subsensitivity of vascular soluble guanylyl cyclase to NO in TGR rats, which could not be restored by addition of superoxide dismutase in vitro. Moreover, changes in soluble guanylyl cyclase activity were only partially reversed by blood pressure reduction alone, but completely normalized after inhibition of the renin–angiotensin system, suggesting that both hypertension per se and an overactive renin–angiotensin system contribute to desensitization of soluble guanylyl cyclase in transgenic hypertensive TGR rats.

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