Vascular \( \beta \)-Adrenergic Receptor Adenylyl Cyclase System From Renin-Transgenic Hypertensive Rats

Gerhard Sitzler, Oliver Zolk, Ulrich Laufs, Martin Paul, Michael Böhm

Abstract—In transgenic rats harboring the mouse \( Ren^{-2d} \) gene [TG(mREN2)27], downregulation of the myocardial \( \beta \)-adrenergic receptor adenylyl cyclase system has been demonstrated previously. Because a reduced vasodilatory reactivity may significantly contribute to hypertension in this model of an activated tissue renin-angiotensin system, the present study investigated alterations of the vascular \( \beta \)-adrenergic receptor adenylyl cyclase system. In freshly harvested aortas from transgenic rats, the activity of adenylyl cyclase was reduced significantly (\( P<.05 \)) in the presence of isoprenaline (10 \( \mu \)mol/L; \(-28 \pm 4.5\% \)), guanosine \( 5' \)-triphosphate, \( 5' \)-guanylylimidodiphosphate [Gpp(NH)p] (100 \( \mu \)mol/L; \(-29 \pm 4.7\% \)), and forskolin (100 \( \mu \)mol/L) with \(-42 \pm 6\% \) and without \(-40 \pm 4.3\% \) MnCl\(_2\). Densities of \( \beta \)-adrenoceptors were similar in both strains. In situ hybridization demonstrated the expression of the transgene in aortic smooth muscle cells. These data indicate a reduced catalyst function as a major contributing factor involved in the maintenance of high blood pressure in TG(mREN2)27. However, in cultivated aortic smooth muscle cells, cAMP production after stimulation with isoprenaline, forskolin, and Gpp(NH)p in the presence or absence of MnCl\(_2\) was not different. Affinities and densities of \( \beta \)-adrenoceptors and amounts of immunohistochemically detected inhibitory and stimulatory G-protein \( \alpha \)-subunits were unchanged. Desensitization after incubation with 10 \( \mu \)mol/L isoprenaline for 72 hours was identical in smooth muscle cells from both strains. Cell cultivation and isoprenaline treatment had no effect on transgene expression. We concluded that in transgenic rats the downregulation of the aortic \( \beta \)-adrenergic adenylyl cyclase system is due to humoral and hemodynamic factors present in vivo rather than to transgenicity itself. 

(Hypertension. 1998;31:1157-1165.)

Key Words: renin-angiotensin system \( \bullet \) adenylyl cyclase \( \bullet \) receptors, adrenergic \( \bullet \) muscle, smooth, vascular \( \bullet \) rats, transgenic

Primary hypertension is thought to be influenced by genetic and environmental factors. Animal models with genetic forms of hypertension can serve as tools to understand genotype-phenotype interactions in this disease. Significant genetic heterogeneity has been demonstrated in polygenic inbred rats.\(^1\) Recently, a transgenic rat model overexpressing a mouse renin gene (mouse \( Ren^{-2d} \)) was generated.\(^2\) It has been characterized by fulminant hypertension and cardiac hypertrophy, low renal and plasma renin activities, and low circulating levels of angiotensin II, thus hypothesized that hypertension in these transgenic animals could at least partly be due to impairment of signal transduction of \( \beta \)-adrenergic receptors in vascular SMCs. In this study, we investigated the expression of the transgene and the function of the \( \beta \)-adrenergic receptor adenylyl cyclase system in freshly harvested aortas. In a second part of our study, we assessed the alterations occurring in cultured SMCs from aortas to determine whether the alterations observed are due to transgenicity itself or to the influence of in vivo or environmental factors.

Methods

Animals

Thirteen heterozygous, male, 12-week-old, transgenic rats [TG (mREN2)27] were obtained from the Max Delbrück Centrum for Molecular Medicine, Berlin, Germany. Age-matched male Sprague-Dawley rats originally obtained from Zentralinstitut für Versuchstierkunde, Hannover, Germany, were used as controls. These rats belong to the strain into which the transgene was originally introduced.\(^2\) The rats were kept in accordance with our institutional guidelines on a standard laboratory animal diet (Altromin) and tap...
Vascular β-Adrenergic System in Renin-Transgenic Rats

Selected Abbreviations and Acronyms

- DMEM = Dulbecco’s modified Eagle’s medium
- Gα-subunit = inhibitory G-protein α-subunit
- Gpp(NH)p = guanosine 5’-triphosphate, 5’-guanylylimidodiphosphate
- Gα-subunit = stimulatory G-protein α-subunit
- PCR = polymerase chain reaction
- RAS = renin-angiotensin system
- SMC = smooth muscle cell

SMC Culture

Rats were killed by cervical dislocation. The abdominal aortas were quickly removed and washed several times in PBS. After connective tissue and fat were trimmed off, aortas were incubated in DMEM containing 1.5 mg/mL collagenase I, 0.5 mg/mL elastase, and trypsin inhibitor for 30 minutes. The adventitia was stripped off, and the remaining media was minced and reincubated with the enzymatic solution for 20 minutes. Tubes were centrifuged for 10 minutes at 484 g. The pellet was seeded in culture wells containing DMEM and 20% fetal calf serum (FCS). Wells were placed in a humidified incubator (5% carbon dioxide, 95% air), and medium was replaced every other day. After reaching confluence, cells were scrubbed and subcultured in DMEM and 5% FCS. Cells in passages 3 to 5 were used for all studies. The identity of cells was confirmed by staining with monoclonal antibodies to α-actin.

Desensitization of β-Adrenergic Pathway in Cultivated SMCs

Cells in passages 3 to 5 at subconfluency were incubated in the presence and absence of 10 μmol/L isoprenaline for 72 hours. Basal and isoprenaline-induced adenylate cyclase activity were determined in particulate washed membrane fractions as described below.

In Situ Hybridization in Freshly Prepared Aortas

In situ hybridization of the mouse Ren-2 transgene was performed according to standard protocols as described elsewhere. In brief, immediately after removal, aortas were embedded in Tissue Tek (Miles Inc) and stored at –80°C. Cryosections (6 μm thick) from transgenic and control aortas were mounted on the same slide to guarantee identical hybridization and washing conditions. Before hybridization, sections were fixed in 4% paraformaldehyde in PBS, and washed twice in PBS according to standard protocols as described elsewhere. In brief, to generate the hybridization postfixed in 4% paraformaldehyde in PBS, and washed twice in PBS according to standard protocols as described elsewhere. In brief, probes, a 600-bp and 0.9% NaCl for 5 minutes each. To generate the hybridization

Harvesting of Fresh Aortas and Preparation of Aortic Media

Aortas were trimmed of connective tissue and fat. The adventitia and the intimal layer were stripped off. The remaining aortic media preparation was used for further studies as described below.

Membrane Preparation for Binding Studies and G-Protein Determinations

Aortic SMCs or media preparations from freshly harvested aortas were homogenized in an ice-cold buffer containing 10 mmol/L Tris-HCl, 1 mmol/L EGTA, 1 mmol/L DTT, pH 7.4, and spun at 484 g for 10 minutes. The supernatant was filtered through cheese-cloth, resuspended in homogenization buffer, and spun at 100 000g for 30 minutes. This step was repeated once. The pellet was resuspended in incubation buffer (50 mmol/L Tris-HCl, 10 mmol/L MgCl₂, pH 7.4) and homogenized in a glass–glass homogenizer for 1 minute. This suspension was centrifuged at 100 000g for 45 minutes. The final pellet was resuspended in incubation buffer and stored at −70°C. Storage did not alter results.

[125I]Iodocyanopindolol Binding Studies

For radioligand experiments, membranes were incubated in incubation buffer (50 mmol/L Tris-HCl, 10 mmol/L MgCl₂, pH 7.4, total volume 250 μL) at 37°C for 60 minutes. This allowed for complete equilibration of receptors and ligand. The reaction was terminated by vacuum filtration through Whatman GF/C filters and immediate washing with ice-cold buffer (three times, 6 mL). All experiments were performed in triplicate. β-Adrenoceptors in membranes from SMCs were studied using increasing concentrations of [125I]iodocyanopindolol (specific activity, 2000 Ci/mmol) for total binding and 3 μmol/L (-)-propranolol to determine nonspecific binding. Remaining radioactivity on the filter was determined in a gamma radiation counter. The approximate total number of β-adrenoceptor binding sites in media from freshly harvested aortas was determined in the presence of 250 μmol/L [125I]iodocyanopindolol corresponding to about 10-fold of the Kᵢ value obtained in membranes from SMCs. In a second series of experiments, β₁- and β₂-adrenoceptor subtypes were determined in competition experiments using CGP 207,12A as β₁-selective antagonist and ICI 118,551 as β₂-selective antagonist in the presence of 50 μmol/L [125I]iodocyanopindolol.

Adenyl Cyclase Activity Determinations

Adenyl cyclase activity was determined according to the methods of Salomon et al with modifications as described recently. In brief, particulate washed membrane fractions (10 000 g sediment) were prepared from homogenates of SMC or preparations of aortic media. The activity of adenylylcyclase was determined in the presence of 50 μmol/L triethanolamine-HCl, 5 mmol/L MgCl₂, 100 μmol/L EGTA,  

Water ad libitum. They were exposed to a 12-hour light/dark cycle at 20°C to 22°C. At the age of 12 weeks, arterial hypertension was fully established in transgenic rats.
1 mmol/L 3-isobutyl-1-methylxanthine (IBMX), 5 mmol/L creatine phosphate, 0.4 mg/mL creatine kinase, and 0.1 mmol/L cAMP at pH 7.4 in a total volume of 100 μL. After 5 minutes of preincubation at 37°C, the reaction was started by addition of membrane suspension. Incubation was stopped after 20 minutes by addition of 500 μL of Zn-acetate (120 mmol/L). After neutralization by 600 μL Na2CO3 (144 mmol/L), tubes were spun at 10,000g for 5 minutes. The supernatant was applied on neutral alumina columns equilibrated with 1 mmol/L Tris-HCl, pH 7.5. [32P]cAMP contents of the effluent were applied on neutral alumina columns equilibrated with ethidium bromide to verify the quality of the RNA preparation. The recovery rate described by Laemmli.15 were detected in a liquid scintillation counter. The recovery rate of [32P]cAMP to the incubation medium ranged from 75% to 92%.

**Immunoblotting**

Immunoblotting was performed as described previously.5,11 In brief, membranes were separated by SDS 10% polyacrylamide electrophoresis before electrophoretic transfer of proteins. Nitrocellulose membranes were incubated with polyclonal antiserum (MB 1) raised in rabbits against the C-terminal decapeptide of retinal transducin α (KENLKDCGLFK) recognizing Gαi3 and Gαo, but not Gαs or Gα12,13 and against the C-terminal decapeptide of Gαs. Blots were stained with peroxidase-conjugated goat anti-rabbit IgG antiserum and visualized by using the enhanced chemiluminiscence assay (ECL-Plus, Amersham-Buchler). After exposure to x-ray film (Kodak X-OMAT AR), signals were quantified by two-dimensional densitometry (Image Quant Densitometric System, Molecular Dynamics).

**mRNA Isolation and PCR**

Cultured SMCs in passage 7 were homogenized. RNA was isolated with RNA-clean (AGS) according to the manufacturer’s protocol to obtain total cellular RNA. This was quantified spectrophotometrically at 260 and 280 nm. Aliquots (2 μg) were electrophoresed through 1.2% agarose/0.6% formaldehyde gels and stained with ethidium bromide to verify the quality of the RNA preparation. Isolated total RNA (2 μg) was reversed transcribed with random primers. The single-stranded cDNA was amplified by PCR with Taq DNA-polymerase (Boehringer Mannheim). Forty cycles were performed under the following conditions: 4 minutes, 94°C; 45 seconds, 94°C; 45 seconds, 60°C; 1 minute, 72°C. The sequences for the mouse Ren-2 transgene sense and antisense primers were 5'-ATG TTT GAC ACG GGC TCC-3' and 5'-AAT GTG GTC AAA GAC -AAT-3', respectively. PCR amplification gave a fragment of 355 bp originating from the mouse Ren-2 transgene.

**Miscellaneous**

Protein was determined according to Lowry et al14 using BSA as the standard. SDS-polyacrylamide gel electrophoresis was performed as described by Lammli.15

**Materials**

Forskolin was donated by Dr Ulrich Metzger (Hoechst AG, Frankfurt, Germany). Gpp(NH)p, ATP, creatine phosphate, and creatine kinase were from Boehringer Mannheim; IBMX was from EGA-Chemie. [3H]Iodocyanopindolol was from Amersham-Buchler. Antiserum against rabbit Gαs was from New England Nuclear. CGP 207.12A and ICI 118.551 were from Ciba-Geigy AG and Imperial Chemical Industries, respectively. PCR products including reverse transcriptase and Taq polymerase were from Boehringer Mannheim. DMEM and FCS were supplied by Gibco BRL. All other compounds used were of the highest analytical grade commercially available. Only deionized and twice-distilled water was used throughout.

**Statistics**

All data are shown as mean±SEM. Statistical significance was estimated by Student’s t test for unpaired observations. A value of P<0.05 was considered to be significant. Binding data were evaluated by Scatchard analysis16 and an iterative curve fitting procedure.17 The monophasicity of competition curves was assessed by means of the Hill equation.18

**Results**

**Adenylyl Cyclase Activity in Freshly Harvested Aortas**

To study a putative effect of transgenicity on signal transduction by adenylyl cyclase, we assessed the β-adrenergic receptor adenylyl cyclase system in both strains. Fig 1 shows adenylyl cyclase activity in freshly harvested aortas without stimulation and after stimulation with increasing concentrations of isoprenaline, Gpp(NH)p, or forskolin (0.01 to 100 mmol/L each, n=3). As assessed in different experiments, the ability of 10 mmol/L isoprenaline to increase cAMP production was reduced significantly in TG(mREN2)27 by 28±4.5% (n=5) compared with controls, indicating a regulation of signal transmission somewhere between receptor level and adenylyl cyclase. The effectiveness of 100 mmol/L Gpp(NH)p, a metabolically stable guanine nucleotide derivative, and 100 mmol/L forskolin, an activator of the catalyst, was reduced by 29±4.7% (n=5) and 40±4.3% (n=5), respectively, in TG(mREN2)27. This suggests involvement of mechanisms at the postreceptor level. However, in some cell systems, forskolin effects depend on Gαi.19 High concentrations of MnCl2 uncouple the catalyst from the effect of GTP-activated G-protein α-subunits20 and thus allow the evaluation of whether impairment of G-protein coupling or of the catalyst itself is involved. Again, a significant reduction of adenylyl cyclase activity by 40±6.3% (n=7) in the presence of MnCl2 alone and by 42±6.0% (n=7) in the presence of forskolin and MnCl2 was observed in TG(mREN2)27 (Fig 1, right lower panel), suggesting a reduced catalyst function as a main contributing factor to downregulation of the β-adrenergic receptor adenylyl cyclase system observed in TG(mREN2)27.

**Density of β-Adrenergic Receptors in Freshly Harvested Aortas**

The density of β-adrenergic binding sites in freshly harvested aortas from transgenic rats and controls was not significantly different [23.6±1.3 fmol/mg protein in TG(mREN2)27 versus 21.9±0.8 fmol/mg protein in controls, n=4, P>0.05], giving further evidence of a heterologous desensitization of the β-adrenergic adenylyl cyclase system in TG(mREN2)27 at the postreceptor level (data not shown).

**In Situ Hybridization in Abdominal Aortas**

Tissue-specific expression of the transgene has been reported in TG(mREN2)27.21 Until now, no data have been available identifying which cells express the transgene in the vascular wall. Therefore, we assessed transgene expression by in situ hybridization. Fig 2 (left photograph) shows a representative slide illustrating the expression of mouse Ren-2α in SMCs (and the endothelium) of abdominal aortas from transgenic rats. In aortas from Sprague-Dawley rats, no expression of the transgene was detected (Fig 2, right photograph). Native rat renin was expressed in aortic walls from both species to an amount close to the detection limit of the assay (data not shown). There was no obvious difference in the grade of expression of native rat renin between both species in this tissue.
Adenylyl Cyclase Activity in Cultivated SMCs

In contrast to experiments in freshly harvested aortas, adenylyl cyclase activity in cultivated SMCs stimulated by increasing concentrations of isoprenaline, Gpp(NH)p, or forskolin was the same in both strains (Fig 3). Because the function of the catalyst was found to be markedly impaired in freshly harvested aortas, the function of the catalyst was evaluated in more detail. Therefore, the effect of 100 μmol/L forskolin

Figure 1. Adenylyl cyclase activity in freshly harvested aortas from transgenic rats [TG(mREN2)27] and controls (SP). Adenylyl cyclase activity after stimulation with increasing concentrations of isoprenaline (upper left), Gpp(NH)p (upper right), and forskolin (lower left) in the absence of MnCl₂ but in the presence of 5 mmol/L MgCl₂. Means±SEM from 3 animals are shown. Lower right, Bar graphs show adenylyl cyclase activity at baseline and after stimulation with 100 μmol/L forskolin in the presence of MnCl₂ (5 mmol/L) but in the absence of MgCl₂. Means±SEM from 7 animals are shown. Ordinate: Adenylyl cyclase activity (in pmol/L cAMP per mg protein/20 min). Abscissa: Condition studied. *Significant reduction vs control (P<.05).

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Figure 2. In situ hybridization for mouse Ren-2 in abdominal aortas from transgenic and control rats. The left photograph illustrates the presence of the transgene in medial SMCs (and in the endothelial layer) in aortas from transgenic rats, whereas no signal was detected in controls (right photograph). Magnification, ×80 each.
was evaluated in presence of MnCl₂ or MnCl₂ plus 100 μmol/L Gpp(NH)p to assess the role of Gpp(NH)p-activated G-protein α-subunits (Fig 3, right lower panel). Again, no difference was found between adenylyl cyclase activities in TG(mREN2)27 and controls, indicating an unchanged activity of the catalyst. Addition of Gpp(NH)p did not further increase the effect of MnCl₂ and forskolin on cAMP production, significantly indicating that uncoupling from G proteins was complete and the activity of the catalyst itself was studied.

**Immunohistochemical Detection and Quantification of Gᵢα- and Gₛα-Subunits in Cultivated SMCs**

To rule out any alteration of the amount of Gᵢα- and Gₛα-subunits in SMCs, G-protein α-subunits were quantified immunochemically. Fig 4 shows representative Western blots and mean±SEM of Gₛα-subunit expression (two isoforms at 52 and 45 kD, left panel) and Gᵢα-subunit expression (right panel) in aortic SMCs. In good agreement with adenylyl cyclase activity in membranes from cultivated SMCs, densitometry revealed identical amounts of Giα- and Gₛα-subunits in controls and TG(mREN2)27.

**β-Adrenergic Receptors in Cultivated SMCs**

Saturation isotherms with [¹²⁵I]iodocyanopindolol, a non-selective β-adrenoceptor antagonist, revealed identical affinities (Kᵦ values: 24.2±4.2 pmol/L in TG(mREN2)27 versus 22.5±4.9 pmol/L in SP; n=6, P>.05) and receptor densities (Bₘₐₓ values: 31.1±7.0 fmol/mg protein in SP versus 35.1±6.5 fmol/mg protein in TG(mREN2)27; n=6, P>.05) in membrane preparations from cultivated SMCs from TG(mREN2)27 and controls (data not shown). Competition experiments in the presence of ICI 118.551, a β₂-adrenoceptor antagonist (Kᵢ, 38±3.8 nmol/L; n=3) showed monophasic competition curves for the β₂-adrenoceptor antagonist (Hill coefficient Nᵢ ranging from 0.83 to 1.12), while CGP 207.12A, a β₁-selective adrenoceptor antagonist had only low affinity (Kᵢ, >1.3 μmol/L; n=3), indicating the (almost) exclusive presence of β₂-adrenoceptors only in SMC membranes in TG(mREN2)27 and controls (Fig 5).

**Desensitization of β-Adrenergic Signal Transduction Pathway in Cultivated SMCs**

To evaluate any difference in desensitization of β-adrenergic signal transduction, SMCs from transgenic and control rats were incubated in the presence and absence of 10 μmol/L isoprenaline for 72 hours. In both strains, significant reduction of isoprenaline-induced cAMP production was detected compared with cells without isoprenaline treatment. However, there were no significant differences in adenylyl cyclase activity in presence of MnCl₂ or MnCl₂ plus 100 μmol/L Gpp(NH)p to assess the role of Gpp(NH)p-activated G-protein α-subunits (Fig 3, right lower panel). Again, no difference was found between adenylyl cyclase activities in TG(mREN2)27 and controls, indicating an unchanged activity of the catalyst. Addition of Gpp(NH)p did not further increase the effect of MnCl₂ and forskolin on cAMP production, significantly indicating that uncoupling from G proteins was complete and the activity of the catalyst itself was studied.

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activity with and without exposure to the agonist between strains (Fig 6). This indicates that the process of desensitization itself is not under the control of the transgene in cultivated SMCs.

Expression of Ren-2\textsuperscript{d} Transgene in Cultivated SMCs

The transgene expression was confirmed by means of PCR up to passage number 7 and in the presence of 10 $\mu$mol/L isoprenaline over 72 hours (Fig 7).

Taken together, our data show a heterologous desensitization of the adenylyl cyclase system in freshly harvested aortas harboring the mouse Ren-2\textsuperscript{d} transgene but not in cultivated aortic SMCs from transgenic rats [TG(mREN2)27].

Discussion

The present study demonstrates for the first time that the $\beta$-adrenergic receptor adenylyl cyclase system is downregulated in aortas from 12-week-old, male, heterozygous, hypertensive transgenic rats harboring the mouse Ren-2\textsuperscript{d} gene [TG(mREN2)27]. In concert with previous studies in the vessel wall of other rat models for hypertension, it has been...
has been demonstrated in aortic tissue from this strain, suggesting a role of the tissue RAS in the downregulation of the β-adrenergic receptor adenylyl cyclase system appears to be very likely. Our results support this notion, since we have detected for the first time the cell type–specific expression of the mouse Ren-2 transgene in medial SMCs of abdominal aortas from transgenic rats by means of in situ hybridization. However, since we detected the mouse Ren-2 transgene in the endothelial layer also and studied intima-free material only, our system may not reflect the in vivo situation completely.

It is well known that blood vessels of hypertensive rats can contain increased norepinephrine concentrations. In rat mesentery arteries, isoprenaline acting via presynaptic β1-adrenoceptors activates the vascular RAS, leading to facilitation of neurotransmission. In good agreement, in human atria facilitation of norepinephrine release from presynaptic nerve terminals by stimulation of presynaptic angiotensin II receptors has been demonstrated. In the long-term, the augmentation of norepinephrine from presynaptic stores by increased local levels of angiotensin II may lead to desensitization of the β-adrenergic adenylyl cyclase system in the vessel wall. In line with this argument, Böhm et al proposed that in hypertrophied myocardium from transgenic animals carrying the Ren-2 gene, this mechanism may induce a reduction of β1-receptor density and an increase of G1 proteins. However, because norepinephrine has a much lower affinity to β1-adrenoceptors than β2-adrenoceptors in aortic tissue, mechanisms other than increased sympathetic overstimulation may be of relevance.

Increased production of angiotensin II is known to be a stimulus for steroid secretion and production in the adrenals of transgenic rats. In deoxycorticosterone-treated rats, desensitization of adenylyl cyclase was observed, which was mainly due to a depressed activity of the catalyt and not to β-adrenoceptor density changes. Therefore, an increase in steroid hormone production in transgenic rats as indicated by an increase in urinary glucocorticoid and mineralocorticoid excretion and a suppression of hypertension by dexamethasone treatment in young transgenic rats may affect not only blood pressure but may also be involved in depressing the activity of the catalyst.

The increase of angiotensin formation in the vessel wall may be not only a primary phenomenon due to transgenicity but also may be due to an activation of other neurohormonal agents, shear stress, and other mechanical factors as well. Therefore, in the second part of our study we evaluated the β-adrenergic receptor adenylyl cyclase system in vascular SMCs from aortic origin. We hypothesized that an effect due to transgenicity itself should be kept unchanged in cultured SMCs, whereas alterations due to in vivo mechanisms should be markedly reduced or lost. We could not detect any differences in β-adrenoceptor densities, β-adrenoceptor affinities, stimulated and unstimulated adenylyl cyclase activities, or in the amounts of G and G proteins between cultured SMCs from both strains. We cannot rule out that alterations of the β-adrenergic receptor adenylyl cyclase system due to cell isolation and cultivation procedures occur. However, identical orders of magnitude of β-adrenoceptor binding sites in membranes from freshly harvested aortas and SMCs as

Figure 7. Expression of the Ren-2 transgene in cultivated SMCs. Representative ethidium bromide–stained agarose gels of a reverse transcription PCR of RNA isolated from SMCs at passage 7 either from transgenic rats or from controls are shown. The 355-bp fragment corresponds to mouse Ren-2 mRNA. Top, Lanes 2, 3, and 5: SMCs from transgenic rats; lane 4: SMCs from nontransgenic rats; lane 5: control without cDNA. Bottom, Lane 2: SMCs from transgenic rats at passage 5 after exposure to 10 μmol/L isoprenaline for 72 hours; lane 3: SMCs from transgenic rats without exposure to isoprenaline; lane 4: control without cDNA. Note that different base standards were used.

demonstrated that in this strain the β-adrenergic effects are mostly transmitted by β2-adrenoceptors. As shown by uncoupling of the catalyticβ2-subunit by manganese ions, the activity of the catalyticβ2-subunit is markedly reduced in TG(mREN2)27, whereas the number of β2-adrenergic binding sites is the same in both species. Thus, the impairment of the catalyst’s function seems to play a major role in the desensitization process of the aortic vessel wall. This is in contrast to recent data obtained in human lymphocytes suggesting impairment of the Gα-protein function in hypertensive subjects.

Heterologous desensitization (ie, downregulation at the postreceptor level) is a very common process in (polygenic) genetic models of hypertension and has been demonstrated in vascular as well as in myocardial tissues. However, in myocardial tissue from renin transgenic rats, regulation induces a reduction of β1-adrenoceptor density, an upregulation of the amount of Gα-subunits, and a mild depression of the activity of the catalytic adenylyl cyclase, whereas β2-adrenoceptor density is not affected. In contrast, in aortic tissue the reduced activity of the catalyst plays a major role, indicating that the mechanisms of desensitization may differ even between different tissue in the same animals.

Gardiner et al suggested that hypertension in this strain may not be due to impairment of cardiac function but to marked peripheral vasoconstriction. Our findings fit well with the assumption that reduced vasodilatory reactivity is due to an impairment of signal transduction mediated by β2-adrenoceptors and may explain, at least partly, the marked increase of afterload observed in these animals.

Because transgenic rats have an activated tissue RAS in several organs, and because an increased expression of the transgene as well as increased local formation of angiotensin...
demonstrated by means of binding studies indicate that, at least on the receptor level, culturing had no major impact on the results of this study. Moreover, in cultivated SMCs from transgenic and control rats exposed to isoproterenol for 72 hours, no evidence of different susceptibility to agonist-induced desensitization of the β-adrenergic signal transduction pathway was seen. In addition, the transgene was detected by means of a PCR procedure after subculturing and after exposure to isoproterenol for 72 hours. These results support the notion that the alterations observed are likely to be due to mechanical or humoral factors present in vivo rather than to effects of transgenicity itself. This finding is in contrast to data provided by Clark et al, who demonstrated a regulation of the β-adrenergic signaling pathway in cultivated SMCs from thoracic aortas from Milan hypertensive rats after several passages, suggesting a genetic determination of these alterations in these strains. However, these authors did not examine signal transduction pathways in freshly harvested aortic tissue, so changes due to subcultivation cannot be ruled out in their experiments. Moreover, in these polygenic models of hypertension, results might be affected by genetic heterogeneity.

As a whole, our results point toward an important role of the cAMP mediation in the regulation of hypertension in TG(mREN2)27. The stimulation of the RAS by cAMP-dependent pathways is well established. This goes well with the recent demonstration of a cAMP-responsive element in the 5'-flanking sequence of the Ren-2 gene. Thus, the depression of the catalytic unit of adenylyl cyclase and the concomitant reduction of intracellular cAMP production demonstrated in freshly harvested aortas from transgenic animals might limit the expression of the mouse renin transgene. As a result, the production of renin and angiotensin in the vessel wall might be reduced. Consequently, the alterations of β-adrenergic signal transduction observed in freshly harvested aortas may represent a negative feedback mechanism countering the induction of hypertension by an activated tissue RAS, leading to increase of angiotensin II in vivo rather than to transgenicity itself.

Acknowledgments
This work was supported by grants from the Deutsche Forschungsgemeinschaft (DFG) and the Fritz-Thyssen Stiftung. Dr Böhm is a recipient of the Gerhard-Hess program (DFG).

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Hypertension. 1998;31:1157-1165
doi: 10.1161/01.HYP.31.5.1157

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