ELSEVIER

European Journal of Pharmacology 341 (1998) 337–341

ejp

Short communication

Signal transduction in cardiac and vascular tissue from normotensive and transgenic hypertensive TGR(mREN2)27 rats

Klaus Witte *, Anke Schnecko, Karsten Perbandt, Stephan Höfer, Björn Lemmer

Faculty of Clinical Medicine Mannheim, Institute of Pharmacology and Toxicology, Ruprecht-Karls-University Heidelberg, Maybachstrasse 14–16, D-68169 Mannheim, Germany

Received 18 August 1997; revised 28 October 1997; accepted 31 October 1997

Abstract

Adenylyl cyclase and soluble guanylyl cyclase activities were measured in cardiac and aortic tissue from transgenic hypertensive TGR(mREN2)27 and normotensive Sprague–Dawley rats. Cardiac basal and stimulated adenylyl cyclase activity was significantly lower in TGR(mREN2)27 than in Sprague–Dawley rats except after uncoupling of G-proteins by Mn^{2+}-ions. Aortic cAMP formation did not differ between both strains, indicating that the disturbance of cardiac adenylyl cyclase activity was due to local rather than systemic factors. Vascular cGMP formation was significantly reduced in TGR(mREN2)27 aortae under basal conditions and after stimulation with sodium nitroprusside, indicating that there is a subsensitive vasodilating second messenger pathway in the transgenic strain. © 1998 Elsevier Science B.V.

Keywords: Guanylyl cyclase, soluble; Adenylyl cyclase; Aorta; Heart; Hypertension

1. Introduction

Changes in the cardiac β-adrenoceptor-adenylyl cyclase system are a common finding in rat models of hypertensive cardiomyopathy (review in: Castellano and Böhm, 1997). Typically, the response to β-adrenergic stimulation of adenylyl cyclase is found to be depressed in diseased myocardium and inhibitory G-proteins are often found to be elevated. It is, however, unclear whether these changes result from local factors, are non-specific consequences of cardiac hypertrophy per se, or reflect systemic overactivation of the sympathetic nervous system. In the present study we measured cardiac as well as vascular adenylyl cyclase activity in transgenic hypertensive TGR(mREN2)27 rats and their normotensive controls. TGR(mREN2)27 rats are characterized by an overactive renin angiotensin-system (Tokita et al., 1994), which leads to fulminant hypertension (Mullins et al., 1990) with typical secondary complications such as vascular damage, nephrosclerosis (Bachmann et al., 1992) and myocardial hypertrophy (Villarreal et al., 1995). In a previously published study, it was shown that cardiac β-adrenergic signal transduction is desensitized in TGR(mREN2)27 rats (Böhm et al., 1994). This could result from increased cardiac angiotensin II concentrations leading to enhanced neuronal noradrenaline release. Based on this hypothesis, it was of interest to study whether comparable alterations occur in the vascular adenylyl cyclase pathway.

The aortic soluble guanylyl cyclase system was studied in TGR(mREN2)27 rats, because impaired nitric oxide-dependent vasodilation has been found in humans and in models of experimental hypertension (review in: Dominiczak and Bohr, 1995), which could involve a reduction in the endothelial release of nitric oxide as well as subsensitivity of the second messenger pathway.

2. Methods

2.1. Animals

Male 10 week old Sprague–Dawley ($n = 35$) and transgenic TGR(mREN2)27 rats ($n = 35$) were obtained from Moellegaard (Skensved). Animals were kept in groups of 5 rats per cage under a 12:12 h light/dark regimen with lights on at 7:00 h, under constant environmental condi-

* Corresponding author. Tel.: +49-621-331067; fax: +49-621-377601; e-mail: witte@rumms.uni-mannheim.de.

tions, and with free access to food and water. After 2 weeks the rats were killed, and heart ventricles and the descending part of the thoracic aorta were dissected, freed from fat and connective tissue, rinsed in ice-cold isotonic saline solution, dried on filter paper, frozen in liquid nitrogen, and stored at −60°C.

2.2. Tissue preparation

Single thoracic aortae with endothelium were homogenized in 1.5 ml of ice-cold GC-buffer (guanylyl cyclase-buffer: Tris–HCl 50 mM, pH 7.4, $MgCl_2$ 5 mM, DL-dithiothreitol 1 mM), using an Ultra-Turrax-homogenizer (IKA, Staufen) at 20 000 rpm, followed by a second homogenization with a Braun–Potter-S glass homogenizer. The resulting suspension was centrifuged for 10 min at $25\,000 \times g$. The supernatant was used for determination of soluble guanylyl cyclase activity; the pellet was resuspended in AC-buffer (Tris–HCl 50 mM, pH 7.4, $MgCl_2$ 5 mM for aortic tissue, 10 mM for cardiac tissue) for measurement of adenylyl cyclase activity. Single ventricles were weighed and immediately homogenized in ice-cold AC-buffer using an Ultra-Turrax-homogenizer (IKA, Staufen) at 20 000 rpm. After 10 min centrifugation at $25\,000 \times g$, the pellets were resuspended in 0.10 ml AC-buffer per mg of tissue. All preparation steps were performed at 4°C to prevent thermal alteration of the enzymes.

2.3. Adenylyl cyclase assay

The formation of cAMP was determined in the presence of the phosphodiesterase-inhibitor 3-isobutyl-1-methylxanthine (IBMX) and an ATP-regenerating system as described (Lemmer and Witte, 1989). Aortic adenylyl cyclase was stimulated by the non-hydrolyzable GTP analogue guanylylimidodiphosphate (GppNHp) 100 μM and by forskolin-7-deacetyl-7-butyryl (forskolin) 100 μM. Cardiac adenylyl cyclase was stimulated by GTP 10 μM, isoprenaline 100 μM (in the presence of GTP 10 μM), GppNHp 100 μM and by forskolin 100 μM. In addition, cardiac cAMP formation was measured in AC-buffer containing Mn^{2+} 10 mM instead of Mg^{2+} 10 mM in order to assess catalytic activity after uncoupling of the G-proteins (Limbird et al., 1979). The amount of cAMP formed was measured by radioassay (TRK 432, Amersham Buchler, Braunschweig).

2.4. Guanylyl cyclase assay

The formation of cGMP by soluble guanylyl cyclase was determined in the presence of IBMX 1 mM and a GTP-regenerating system containing GTP 0.5 mM, phosphocreatine 10 mM, and creatine phosphokinase 0.1 mg/ml as described in detail (Witte et al., 1995). The enzyme was stimulated by addition of the nitric oxide donor sodium nitroprusside 10 and 100 μM. The amount of cGMP formed was measured by radioimmunoassay (TRK 500, Amersham Buchler, Braunschweig).

2.5. Protein concentration

The protein content of the supernatant and pellet of the aortic tissue preparation was determined by using the Coomassie® Plus assay (Pierce, oud-Beijerland). The protein content of the cardiac tissue preparation was measured according to Lowry et al. (1951). Bovine serum albumin (Sigma, Deisenhofen) was dissolved in the respective buffer for use as standard.

2.6. Statistics

Differences between strains were tested by using the non-parametric Mann–Whitney *U*-test. BiAS 5.0 (Ackermann, 1997) was used as statistics software. Data are shown as means ± S.D. unless otherwise indicated.

Table 1
Activities of adenylyl and guanylyl cyclase in cardiac and aortic tissue from Sprague–Dawley and transgenic TGR(mREN2)27 rats

	n	Cardiac adenylyl cyclase (pmol/mg/min)					
		basal	GTP 10 μM	IPN 100 μM	GppNHp 100 μM	FOR 100 μM	Mn^{2+} 10 mM
SPRD	(33)	63.7 ± 15.5	82.4 ± 19.1	193.0 ± 7.0	237.6 ± 70.7	1212.1 ± 266.1	517.0 ± 110.4
TGR	(33)	44.2 ± 16.1	62.9 ± 19.9	153.6 ± 46.7	176.4 ± 47.7	939.0 ± 236.4	534.9 ± 173.4
U-test		$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	n.s.
	n	Aortic adenylyl cyclase (pmol/mg/min)			Aortic soluble guanylyl cyclase (pmol/mg/min)		
		basal	GppNHp 100 μM	FOR 100 μM	basal	SNP 10 μM	SNP 100 μM
SPRD	(35)	103.8 ± 34.4	298.0 ± 106.2	3170.1 ± 1325.2	23.9 ± 10.6	583.6 ± 284.5	1679.5 ± 488.7
TGR	(35)	97.5 ± 15.4	277.1 ± 61.7	2846.9 ± 769.3	14.8 ± 5.1	305.9 ± 177.3	792.0 ± 352.3
U-test		n.s.	n.s.	n.s.	$P < 0.001$	$P < 0.001$	$P < 0.001$

Enzyme activities (pmol/mg/min) represent mean value ± S.D., SPRD = Sprague–Dawley rats, TGR = TGR(mREN2)27 rats.
IPN = isoprenaline (in the presence of GTP 10 μM), FOR = forskolin-7-deacetyl-7-butyryl, SNP = sodium nitroprusside, *U*-test = non-parametric Mann–Whitney *U*-test, n.s. = not significant.

3. Results

3.1. Cardiac tissue

Basal and stimulated rates of cAMP formation were significantly lower in ventricles from TGR(mREN2)27 than in ventricles from Sprague–Dawley rats, except after uncoupling of G-proteins by Mn^{2+}-ions (Table 1). Net increases in adenylyl cyclase activity produced by isoprenaline 100 μM (minus cAMP formation in the presence of GTP) were 110.6 ± 33.6 and 90.7 ± 33.7 pmol/mg/min in Sprague–Dawley and TGR(mREN2)27 rats, representing a relative stimulation (*x*-fold the basal activity in the presence of GTP) of 2.4 ± 0.5 and 2.5 ± 0.5, respectively. While the absolute stimulation of adenylyl cyclase by isoprenaline was significantly ($P < 0.01$) reduced in hypertensive rats, the relative increase in enzyme activity did not differ between the strains. Activation of G-proteins by GppNHp 100 μM induced net increases over basal cAMP formation of 173.9 ± 59.6 pmol/mg/min (3.8 ± 0.6-fold basal) in Sprague–Dawley and 132.2 ± 39.0 pmol/mg/min (4.2 ± 1.1-fold basal) in TGR(mREN2)27 rats. As observed with isoprenaline, the absolute effect of GppNHp was reduced ($P < 0.001$) in TGR(mREN2)27 ventricles, whereas the relative increase did not differ from that of normotensive controls.

In contrast, the net increase in cAMP formation in the presence of Mn^{2+}-ions was not different between the strains, but the stimulation factor was significantly ($P < 0.001$) greater in TGR(mREN2)27 (13.2 ± 5.7-fold basal) than in Sprague–Dawley controls (8.3 ± 1.4-fold basal).

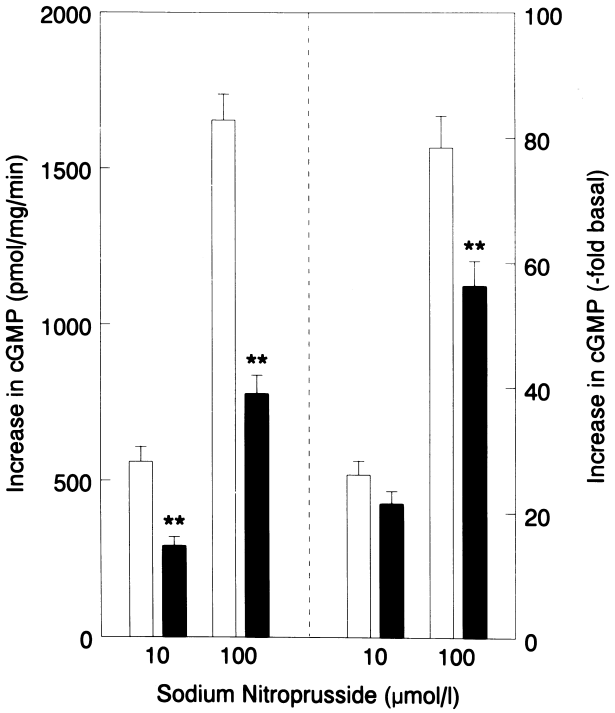

Fig. 1. Sensitivity of soluble guanylyl cyclase to stimulation by the nitric oxide-donor sodium nitroprusside in aortic tissue from normotensive Sprague–Dawley ($n = 35$, open bars) and transgenic hypertensive TGR(mREN2)27 rats ($n = 35$, closed bars). Data (means ± SEM) are expressed as net increase in cGMP formation (left ordinate) and as *x*-fold stimulation (right ordinate). Absolute and relative increases in guanylyl cyclase activity were lower in tissue from the hypertensive strain. $^{**}P < 0.001$, Mann–Whitney *U*-test.

3.2. Aortic tissue

Basal rates of aortic cAMP formation were 103.8 ± 34.4 pmol/mg/min in Sprague–Dawley ($n = 35$) and 97.5 ± 15.4 pmol/mg/min in TGR(mREN2)27 rats ($n = 35$) and did not differ between the strains. Addition of GppNHp and forskolin resulted in a comparable stimulation of adenylyl cyclase activity in aortae from both strains (Table 1). Relative increases in cAMP formation were 2.9 and 2.8-fold by GppNHp 100 μM, and 30.5 and 29.2-fold by forskolin 100 μM in Sprague–Dawley and TGR(mREN2)27 rats, respectively.

Basal rates of cGMP formation by soluble guanylyl cyclase were 23.9 ± 10.6 pmol/mg/min in Sprague–Dawley aortae ($n = 35$) and 14.8 ± 5.1 pmol/mg/min in tissue from TGR(mREN2)27 rats ($n = 35$), being significantly lower in the hypertensive strain ($P < 0.001$). In the presence of sodium nitroprusside 10 and 100 μM tissue from transgenic rats showed significantly lower rates of cGMP formation (Table 1). The effects of stimulation with sodium nitroprusside, calculated as net increase (stimulated minus basal activity) and as relative increase (*x*-fold the basal activity), were also significantly smaller in aortic tissue from TGR(mREN2)27 rats (Fig. 1).

4. Discussion

In the present study we observed a reduced basal as well as stimulated adenylyl cyclase activity in cardiac tissue from TGR(mREN2)27 rats, while in aortic tissue from the same rats basal and stimulated cAMP formation did not differ between transgenic rats and their normotensive controls. In addition, vascular cGMP formation by soluble guanylyl cyclase and its stimulation by the nitric oxide donor sodium nitroprusside were found to be reduced in the hypertensive strain, indicating a possible involvement of the nitric oxide-cGMP pathway in the fulminant hypertension of TGR(mREN2)27 rats.

Our observation of a reduced cardiac cAMP formation in TGR(mREN2)27 rats is in agreement with an earlier study (Böhm et al., 1994) showing a desensitized myocardial adenylyl cyclase system, down-regulation of β_1-adrenoceptors and increased $G_{i\alpha}$-protein in this strain of rats. While Böhm et al. (1994) additionally found a depressed catalytic activity of adenylyl cyclase, the present

study does not support a disturbed function of the enzyme itself, because in the presence of Mn^{2+}-ions no strain-dependent differences were observed. Moreover, the relative increase in cAMP formation in the presence of Mn^{2+}-ions was significantly greater in TGR(mREN2)27 than in Sprague–Dawley rats, being consistent with a tonic inhibition of adenylyl cyclase by G_i-proteins in the presence of Mg^{2+}-ions, which is abolished after uncoupling of G-proteins by Mn^{2+}-ions.

Since the aortic adenylyl cyclase did not show strain-dependent differences, the disturbed cardiac cAMP formation is probably due to local rather than systemic factors. A simple 'dilution' of the cardiac enzyme by increased myocardial mass in transgenic hypertensive rats is unlikely to play a role, because in the presence of Mn^{2+}-ions the rates of cAMP formation did not differ between the strains. Facilitation of noradrenaline release from cardiac neurons by the higher concentrations of angiotensin II in TGR(mREN2)27 rats could be one pathogenetic factor leading to desensitization of the myocardial adenylyl cyclase pathway. This hypothesis is supported by the previous observation of a reduced myocardial content of noradrenaline as well as neuropeptide Y in TGR(mREN2)27 ventricles (Böhm et al., 1994), indicating an increased turnover of the catecholamine and the co-released neuropeptide. Furthermore, it has been shown that the amount of renin mRNA is significantly higher in left ventricles from 13–14 week old transgenic rats than in age-matched normotensive Sprague–Dawley and spontaneously hypertensive rats (Pinto et al., 1997). Thus, an activated cardiac renin-angiotensin system in TGR(mREN2)27 rats could lead to high local concentrations of angiotensin II, which increase cardiac noradrenaline turnover and finally result in a desensitized adenylyl cyclase system.

Our observation of a reduced vascular guanylyl cyclase activity in aortic tissue from TGR(mREN2)27 rats points to a possible involvement of this vasodilating second messenger system in the marked hypertension in this strain. In human hypertension several studies demonstrated impaired nitric oxide-dependent vasodilation (review in: Dominiczak and Bohr, 1995), which was most probably due to impaired basal (Forte et al., 1997) and stimulated (Panza et al., 1995) synthesis of nitric oxide by the endothelium. Since vasodilator responses to sodium nitroprusside do not differ between normotensive and hypertensive subjects (Panza et al., 1995), a disturbed activity of soluble guanylyl cyclase, as observed in TGR(mREN2)27 aortae in the present study, is unlikely to contribute to human hypertensive disease. In rat models of hypertension, divergent findings have been reported concerning the role of nitric oxide in blood pressure regulation. Thus, the vascular release of nitric oxide does not seem to differ between spontaneously hypertensive rats (SHR) and their normotensive Wistar–Kyoto controls, whereas in stroke-prone SHR, mineralocorticoid and renal hypertensive rats a reduced nitric oxide synthesis has been reported (review in: Dominiczak and Bohr, 1995). Interestingly, it has been shown that endothelium-dependent relaxation of aortic rings (Konishi and Su, 1983; Lüscher and Vanhoutte, 1986) and carotid artery preparations (Lüscher et al., 1988) is reduced in SHR rats despite an unchanged release of nitric oxide in response to acetylcholine (Sawada et al., 1994). These findings point to a disturbance downstream of nitric oxide synthesis, which could involve subsensitivity of soluble guanylyl cyclase. Unfortunately, studies on vascular nitric oxide synthesis have not been done in transgenic hypertensive rats. However, Arribas et al. (1994) reported that removal of the endothelium from TGR(mREN2)27 aorta segments resulted in greater contractile responses compared to those of normotensive controls, indicating an increased tonic release of nitric oxide in the hypertensive strain. Based on this observation, one may speculate that the reduced activity of aortic soluble guanylyl cyclase in TGR(mREN2)27 rats results from agonist-dependent desensitization. However, the vasorelaxation elicited by acetylcholine is unimpaired in aorta (Arribas et al., 1994) and coronary artery (Tschudi et al., 1994) from transgenic hypertensive rats. In the light of these discrepant findings and without data for antihypertensive-treated TGR(mREN2)27 rats, it remains an open question whether subsensitivity of aortic guanylyl cyclase is causally linked to hypertension in this strain of rats or represents a non-specific secondary phenomenon. Future studies will have to characterize the definite role of the nitric oxide-guanylyl cyclase system in hypertension of TGR(mREN2)27 rats.

In conclusion, the results of the present study demonstrate an impaired sensitivity of vascular soluble guanylyl cyclase to nitric oxide in TGR(mREN2)27 rats, which may contribute to the fulminant hypertension in this rat strain. The disturbed cardiac adenylyl cyclase system is unlikely to represent a general disturbance of the cAMP pathway in TGR(mREN2)27 rats, because aortic cAMP formation did not differ between transgenic and control animals.

Acknowledgements

This study was supported in part by the 'Paul und Cilli Weill-Stiftung'.

References

Ackermann, H., 1997. Biometrische Analyse von Stichproben. 7th ed. Epsilon Verlag, Hochheim, Darmstadt.

Arribas, S., Sanchez-Ferrer, C.F., Peiro, C., Ponte, A., Salaices, M., Marin, M., 1994. Functional vascular renin-angiotensin system in hypertensive transgenic rats for the mouse renin gene Ren-2. Gen. Pharmac. 25, 1163–1170.

Bachmann, S., Peters, J., Engler, E., Ganten, D., Mullins, J., 1992. Transgenic rats carrying the mouse renin gene-morphological characterization of a low-renin hypertension model. Kidney Int. 41, 24–36.

Böhm, M., Moll, M., Schmid, B., Paul, M., Ganten, D., Castellano, M., Erdmann, E., 1994. β-Adrenergic neuroeffector mechanisms in cardiac hypertrophy of renin transgenic rats. Hypertension 24, 653–662.

Castellano, M., Böhm, M., 1997. The cardiac β-adrenoceptor-mediated signaling pathway and its alterations in hypertensive heart disease. Hypertension 29, 715–722.

Dominiczak, A.F., Bohr, D.F., 1995. Nitric oxide and its putative role in hypertension. Hypertension 25, 1202–1211.

Forte, P., Copland, M., Smith, L.M., Milne, E., Sutherland, J., Benjamin, N., 1997. Basal nitric oxide synthesis in essential hypertension. Lancet 349, 837–842.

Konishi, M., Su, C., 1983. Role of endothelium in dilator responses of spontaneously hypertensive rat arteries. Hypertension 5, 881–886.

Lemmer, B., Witte, K., 1989. Circadian rhythm of the in vitro stimulation of adenylate cyclase in rat heart tissue. Eur. J. Pharmacol. 159, 311–315.

Limbird, L.E., Hickey, A.R., Lefkowitz, R.J., 1979. Unique uncoupling of the frog erythrocyte adenylyl cyclase system by manganese ions. J. Biol. Chem. 254, 2677–2683.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265–275.

Lüscher, T.F., Vanhoutte, P.M., 1986. Endothelium-dependent contractions to acetylcholine in the aorta of the spontaneously hypertensive rat. Hypertension 8, 344–348.

Lüscher, T.F., Diederich, D., Weber, E., Vanhoutte, P.M., Bühler, F.R., 1988. Endothelium-dependent responses in carotid and renal arteries of normotensive and hypertensive rats. Hypertension 11, 573–578.

Mullins, J.J., Peters, J., Ganten, D., 1990. Fulminant hypertension in transgenic rats harbouring the mouse Ren-2 renin gene. Nature 344, 541–544.

Panza, J.A., Garcia, C.E., Kilcoyne, C.M., Quyyumi, A.A., Cannon, R.O., 1995. Impaired endothelium-dependent vasodilation in patients with essential hypertension. Circulation 91, 1732–1738.

Pinto, Y.M., Buikema, H., van Gilst, W.H., Scholtens, E., van Geel, P.P., de Graeff, P.A., Wagner, J., Paul, M., 1997. Cardiovascular end-organ damage in Ren-2 transgenic rats compared to spontaneously hypertensive rats. J. Mol. Med. 75, 371–377.

Sawada, Y., Sakamaki, T., Nakamura, T., Sato, K., Ono, Z., Murata, K., 1994. Release of nitric oxide in response to acetylcholine is unaltered in spontaneously hypertensive rats. J. Hypertens. 12, 745–750.

Tokita, Y., Franco-Saenz, R., Reimann, E.M., Mulrow, P.J., 1994. Hypertension in the transgenic rat TGR(mRen-2)27 may be due to enhanced kinetics of the reaction between mouse renin and rat angiotensinogen. Hypertension 23, 422–427.

Tschudi, M.R., Noll, G., Arnet, U., Novosel, D., Ganten, D., Lüscher, T.F., 1994. Alterations in coronary artery vascular reactivity of hypertensive Ren-2 transgenic rats. Circulation 89, 2780–2786.

Villarreal, F.J., Mackenna, D.A., Omens, J.H., Dillmann, W.H., 1995. Myocardial remodeling in hypertensive Ren-2 transgenic rats. Hypertension 25, 98–104.

Witte, K., Schnecko, A., Zuther, P., Lemmer, B., 1995. Contribution of the nitric oxide-cGMP system to circadian regulation of blood pressure in normotensive Wistar–Kyoto rats. Cardiovasc. Res. 30, 682–688.