



# Sympathectomy-induced blood pressure reduction in adult normotensive and hypertensive rats is counteracted by enhanced cardiovascular sensitivity to vasoconstrictors

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## Abstract

The effect of chemical sympathectomy on cardiovascular parameters and the compensatory role of adrenal hormones, the renin–angiotensin system, and cardiovascular sensitivity to vasoconstrictors were studied in spontaneously hypertensive rats (SHRs) and normotensive Wistar-Kyoto (WKY) rats. Sympathectomy was induced in 20-week-old rats by daily intraperitoneal guanethidine administration (30 mg/kg b.w.) for 2 weeks. Basal blood pressure (BP), heart rate (HR), and restraint stress-induced cardiovascular changes were measured by radiotelemetry. The BP response to catecholamines was determined in rats with implanted catheters. Sympathectomy decreased BP only transiently, and after 14-day guanethidine treatment, BP returned to basal values in both strains. Sympathectomy permanently lowered HR, improved baroreflex sensitivity, and decreased the low-frequency domain of systolic blood pressure variability (a marker of vascular sympathetic activity). Guanethidine also attenuated the BP and HR responses to restraint stress. On the other hand, the BP response to catecholamines was augmented in sympathectomized rats, and this was not due to the de novo synthesis of vascular adrenergic receptors. Sympathectomy caused adrenal enlargement, enhanced the expression of adrenal catecholamine biosynthetic enzymes, and elevated plasma adrenaline levels in both strains, especially in WKY rats. Guanethidine also increased the plasma levels of aldosterone and corticosterone in WKY rats only. In conclusion, sympathectomy produced a transient decrease in BP, a chronic decrease in HR and improvement in baroreflex sensitivity. The effect of sympathectomy on BP was counteracted by increased vascular sensitivity to catecholamines in WKY rats and SHRs and/or by the enhanced secretion of adrenal hormones, which was more pronounced in WKY rats.

**Keywords** Guanethidine · Catecholamines · Blood pressure response · Vascular wall innervation · Adrenal medulla

## Introduction

The sympathetic nervous system (SNS) plays an essential role in the regulation of blood pressure (BP) and in the development of human and experimental hypertension [1].

In adult spontaneously hypertensive rats (SHRs), SNS hyperactivity [2], denser vascular sympathetic innervation [3, 4], and numerous other SNS alterations have been described (for a review see refs. [5, 6]). On the other hand, we demonstrated a decrease in the expression of enzymes associated with catecholamine biosynthesis in the sympathetic ganglia and adrenal medulla during hypertension development in SHRs [4], which might compensate for sympathetic hyperactivity in this strain.

The destruction of peripheral sympathetic neurons (chemical sympathectomy) in neonatal SHRs attenuates the development of hypertension, but moderate BP elevation persists in SHRs compared to normotensive Wistar-Kyoto (WKY) rats [7]. This strain difference can be abolished by sympathectomy combined with adrenal demedullation [8] or with  $\alpha_1$ -adrenergic blockade [9]. The

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effect of chronic sympathectomy in adult rats with established hypertension is studied less frequently than that in young animals, although the treatment of adult animals offers a better model of the treatment of human essential hypertension. Sympathectomy of 10-week-old SHR by 6-hydroxydopamine moderately lowers BP and attenuates cardiovascular responses to the noradrenaline-releasing agent tyramine [10]. However, sympathectomy induced by 6-hydroxydopamine in hypertensive animals is incomplete, and the rapid restoration of adrenergic nerve function may occur after the end of the treatment [11, 12]. An effective method of permanent sympathectomy of adult rats is the chronic administration of guanethidine [13, 14]. Guanethidine, which is actively incorporated into peripheral noradrenergic terminals, decreases catecholamine content in the vascular wall and the low-frequency component of systolic blood pressure variability (SBPV; a marker of vascular sympathetic activity) [15], but it does not affect the chromaffin cells of the adrenal medulla or central catecholaminergic cells [13, 16].

The effects of sympathectomy on BP can be counteracted by several compensatory mechanisms (e.g., higher catecholamine release from the adrenal medulla, the increased involvement of the renin–angiotensin system or the enhanced responsiveness of vascular smooth muscle cells to available vasoconstrictors), as reported previously [8, 17, 18]. Therefore, we tested the hypothesis that the BP-lowering effect of chemical sympathectomy in adult rats is counteracted by the above compensatory mechanisms and that the involvement of these compensatory mechanisms may be different in hypertensive SHR and normotensive WKY rats.

## Methods

The methods are described in detail in the Supplementary Information.

### Animals and sympathectomy

Chemical sympathectomy was performed in male SHR and normotensive WKY rats between the ages of 20 and 22 weeks by a daily intraperitoneal administration of guanethidine (30 mg/kg body weight (b.w.)) for 2 weeks [15, 19]; the measurements were performed on the 14th day of guanethidine treatment. Control (CTRL) rats were injected with saline. Animals were kept under standard laboratory conditions (temperature  $23 \pm 1^\circ\text{C}$ , 12-h light–dark cycle, Altromin pellet diet, and tap water ad libitum). All experimental procedures were approved by the *Ethical Committee of the Institute of Physiology, Czech Academy of Sciences* and conformed to the *European*

*Convention on Animal Protection and Guidelines on Research Animal Use*.

### Radiotelemetry

Adult SHR and WKY rats were implanted with telemetry transmitters (model HD-S10, Data Sciences International, New Brighton) as previously described [15, 20]. After a 10-day recovery period, basal BP and HR were evaluated in freely moving rats, and thereafter, 14-day guanethidine treatment was started. Power spectral analysis of SBPV was used to evaluate the effects of guanethidine on the low-frequency (LF: 0.2–0.75 Hz), and high-frequency (HF: 0.75–4 Hz) components of SBPV as previously described [15]. We also evaluated the effect of restraint stress on cardiovascular parameters. Baroreflex function was determined by the spontaneous sequence technique [21] using Hemolab software (ver. 21.0) programmed by Harald Stauss.

### Cardiovascular response to vasoactive agents

One day prior to BP measurement, catheters were inserted into the left carotid artery and jugular vein of the rats under isoflurane anesthesia. BP and HR were measured in conscious rats kept in small transparent cages (partially restrained) after 24 h of recovery using the PowerLab system (ADInstruments, Bella Vista) between 8 a.m. and 11:30 a.m. [15, 22]. In one group of rats, noncumulative doses of noradrenaline (0.001–10  $\mu\text{g}/\text{kg}$  b.w.) or adrenaline (0.001–10  $\mu\text{g}/\text{kg}$  b.w.) were administered intravenously. In the second group of rats, single doses of captopril (10 mg/kg b.w.) and pentolinium (5 mg/kg b.w.) were injected intravenously into conscious WKY rats and SHR [23], followed by noncumulative doses of Ang II (0.1–100 ng/kg b.w.). The extent of sympathectomy was verified by the administration of the catecholamine-releasing agent tyramine (100  $\mu\text{g}/\text{kg}$  b.w.) after the end of the dose response experiments.

### Blood and tissue sampling

Rats were anesthetized by isoflurane (2.5%), and the blood, the adrenal glands, the femoral arteries, and vascular smooth muscle samples from the thoracic aorta were collected and processed for further use.

### Histochemical visualization of monoamines (SPG method)

The histochemical visualization of monoamines in the femoral arteries was performed according to the protocol of de la Torre and Surgeon [24] as described previously [19]. The quantification was performed as described in our previous paper [4].

## Catecholamines and corticosteroid measurements

The concentrations of catecholamines (noradrenaline and adrenaline) in the plasma and adrenal homogenates were measured using 3-CAT Research ELISA (LDN, Nordhorn). Plasma normetadrenaline and metadrenaline levels were measured with 2-MET Plasma ELISA Fast Track (LDN). Plasma corticosterone and aldosterone levels were measured using a corticosterone rat/mouse ELISA kit (LDN) and an aldosterone ELISA kit (LDN).

## Laser capture microdissection

A total area of 1 mm<sup>2</sup> of the adrenal medulla was dissected using the Leica LMD 6000 Laser Microdissection System (Leica Microsystems, Wetzlar) to measure messenger RNA (mRNA) expression.

## RNA isolation, reverse transcription, and quantitative real-time PCR

Total RNA from smooth muscle samples was isolated using the RNeasy Fibrous Tissue Mini Kit (Qiagen, Hilden). Laser capture microdissected samples of the adrenal medulla were isolated with the Quick-RNA<sup>TM</sup> MicroPrep kit (Zymo Research, Irvine). Genomic DNA was removed with RNase-free DNase I (Qiagen). RNA from the vascular smooth muscle cells was transcribed into complementary DNA (cDNA) by the High-Capacity cDNA Reverse Transcription kit (Life Technologies, Carlsbad). RNA from the adrenal medulla was transcribed using the more sensitive SuperScript<sup>®</sup> VILO<sup>TM</sup> cDNA synthesis kit (Thermo Fisher Scientific, Waltham). The gene expression was determined on a LightCycler<sup>®</sup> 480 System (Roche, Basel,) using HOT FIREPol<sup>®</sup> Probe qPCR Mix Plus (SolisBioDyne, Tartu) and TaqMan<sup>®</sup> Gene Expression Assays (Life Technologies). A list of the TaqMan<sup>®</sup> Gene Expression Assays that were used is available in Supplementary Table 1. The expression data were processed as previously described [4]. Reference genes (*Gapdh* and *Hprt1* in the adrenal medulla and *Hmbz* and *Ywhaz* in the aortic smooth muscles) were selected as described in our previous study [25].

## Statistics

The data are expressed as the means ± SEM. The normality of distribution was tested by the Shapiro–Wilk test. Statistical significance in the radiotelemetry experiment was determined by two-way repeated measures ANOVA. The BP responses to catecholamines and Ang II were analyzed by three-way ANOVA. Other experiments were analyzed by two-way ANOVA. Bonferroni post-hoc test was used in the case of a significant interaction between

factors. The differences were considered to be significant at  $p < 0.05$ .

## Results

### Body weight and adrenal weight

The body weight of SHR rats was significantly lower than that of WKY rats before guanethidine treatment ( $312 \pm 3$  vs.  $343 \pm 7$  g;  $p < 0.001$ ). CTRL animals gained a small amount of body weight, while 14-day guanethidine treatment prevented such a change in sympathectomized (SYMPX) SHR rats and even caused a body weight loss in WKY rats (CTRL SHR rats,  $14 \pm 3$  g; CTRL WKY rats,  $6 \pm 3$  g; SYMPX SHR rats,  $1 \pm 3$  g; SYMPX WKY rats,  $-21 \pm 2$  g; interaction between strain and treatment:  $F_{1,85} = 8.8$ ,  $p < 0.01$ ). The relative weight of the adrenal glands was significantly greater in CTRL SHR rats than in WKY rats. Sympathectomy increased the adrenal weight and abolished this strain difference (CTRL SHR rats,  $13.8 \pm 0.3$  mg/100 g; CTRL WKY rats,  $11.1 \pm 0.2$  mg/100 g; SYMPX SHR rats,  $15.5 \pm 0.6$  mg/100 g; SYMPX WKY rats,  $15.2 \pm 0.4$  mg/100 g; interaction between strain and treatment:  $F_{1,27} = 9.2$ ,  $p < 0.01$ ).

### Cardiovascular parameters measured by radiotelemetry

We used radiotelemetry to compare changes in BP, heart rate (HR) and activity over time in adult freely moving SHR rats and WKY rats sympathectomized by guanethidine (Table 1 and 2). The mean arterial pressure (MAP), HR, activity, and the LF SBPV in CTRL rats of both strains were higher during the dark phase (active period) than during the light phase (resting period). Untreated SHR rats had higher MAP than WKY rats during both the light and dark phases. After 3 days of guanethidine treatment, the MAP was lowered in both strains, and the effect was more pronounced during the dark phase. However, the MAP returned back to the control level after 14 days of guanethidine treatment, and a small persistent decrease in the MAP was found only in WKY rats during the dark phase. SHR rats and WKY rats had similar HRs before the onset of guanethidine treatment (Table 1). HR was lowered following 3 days of guanethidine administration during both the light and dark phases in both strains, and the effect persisted until the 14th day of guanethidine treatment. However, during the dark phase, the HR of SYMPX SHR rats was higher than that of SYMPX WKY rats. SHR rats were more active than WKY rats during the dark phase, and sympathectomy decreased the activity of both strains during the dark phase (Table 1). The LF SBPV (a marker of vascular sympathetic activity) was higher in SHR rats than in WKY rats, and the difference was

**Table 1** Mean arterial pressure (MAP), heart rate (HR) and animal activity in control (CTRL) and sympathectomized (SYMPX) WKY and SHR rats

	MAP (mmHg)		HR (bpm)		Animal activity (AU)	
	Light	Dark	Light	Dark	Light	Dark
<b>WKY</b>						
CTRL D0	104 ± 2	109 ± 1	291 ± 6	343 ± 5	1.7 ± 0.1	4.1 ± 0.2
SYMPX D3	96 ± 1 <sup>†</sup>	98 ± 1 <sup>†</sup>	258 ± 4 <sup>†</sup>	272 ± 4 <sup>†</sup>	2.6 ± 0.4	3.7 ± 0.3
SYMPX D14	105 ± 2	103 ± 2 <sup>†</sup>	267 ± 3 <sup>†</sup>	270 ± 3 <sup>†</sup>	2.2 ± 0.4	2.9 ± 0.2 <sup>†</sup>
<b>SHR</b>						
CTRL D0	157 ± 3*	164 ± 3*	287 ± 5	346 ± 4	2.6 ± 0.3	7.3 ± 0.5*
SYMPX D3	143 ± 2* <sup>†</sup>	145 ± 3* <sup>†</sup>	267 ± 4 <sup>†</sup>	293 ± 3* <sup>†</sup>	3.5 ± 0.6	5.5 ± 0.4* <sup>†</sup>
SYMPX D14	161 ± 4*	164 ± 4*	270 ± 3 <sup>†</sup>	297 ± 8* <sup>†</sup>	3.0 ± 0.6	5.3 ± 0.5* <sup>†</sup>
<b>Factor</b>						
Strain	$F_{1,24} = 272$ $p < 0.001$	$F_{1,24} = 297$ $p < 0.001$	$F_{1,24} = 0.3$ NS	$F_{1,24} = 9.3$ $p < 0.01$	$F_{1,24} = 2.8$ NS	$F_{1,24} = 26.2$ $p < 0.001$
Treatment	$F_{2,24} = 45.2$ $p < 0.001$	$F_{2,24} = 79.2$ $p < 0.001$	$F_{2,24} = 54.6$ $p < 0.001$	$F_{2,24} = 211$ $p < 0.001$	$F_{2,24} = 5.5$ $p < 0.05$	$F_{2,24} = 60.3$ $p < 0.001$
Interaction	$F_{2,24} = 4.4$ $p < 0.05$	$F_{2,24} = 14.7$ $p < 0.001$	$F_{2,24} = 3.3$ $p = 0.056$	$F_{2,24} = 6.8$ $p < 0.01$	$F_{2,24} = 0.01$ NS	$F_{2,24} = 13.2$ $p < 0.001$

Cardiovascular parameters were measured by radiotelemetry in freely-moving animals during the light and dark phase. Data are the means ± SEM of 1 day before guanethidine treatment (D0), the third day of guanethidine treatment (D3) and the 14th day of guanethidine treatment (D14).  $n = 6-7$  for each group. Statistical significance was computed by repeated measures two-way ANOVA, in the case of interaction Bonferroni post-hoc test was performed; \* $p < 0.05$  vs WKY rats; <sup>†</sup> $p < 0.05$  vs CTRL rats (D0) of the same strain.

more pronounced during the dark phase (Table 2). The LF SBPV was markedly decreased in both strains after 3 days and 14 days of guanethidine administration, demonstrating the persistent effect of sympathectomy. This finding is consistent with the results obtained from the visualization of monoamines in the sympathetic innervation of the femoral arteries (Fig. 1a). The quantification of the fluorescent signal (Fig. 1b) revealed a strain difference in vascular sympathetic innervation, which was abolished by sympathectomy (interaction between strain and treatment:  $F_{1,31} = 9.4$ ,  $p < 0.01$ ). The fluorescent signal was ~2.5-fold higher in CTRL SHRs than in WKY rats ( $p < 0.001$ ). Fourteen days of guanethidine treatment reduced the fluorescent signal in both strains (−92% in SYMPX SHRs and −88% in SYMPX WKY rats;  $p < 0.001$  for SHRs and  $p < 0.01$  for WKY rats). The HF SBPV (a marker of cardiac sympathetic activity) was higher in CTRL SHRs, and sympathectomy decreased the HF SBPV in both strains (Table 2).

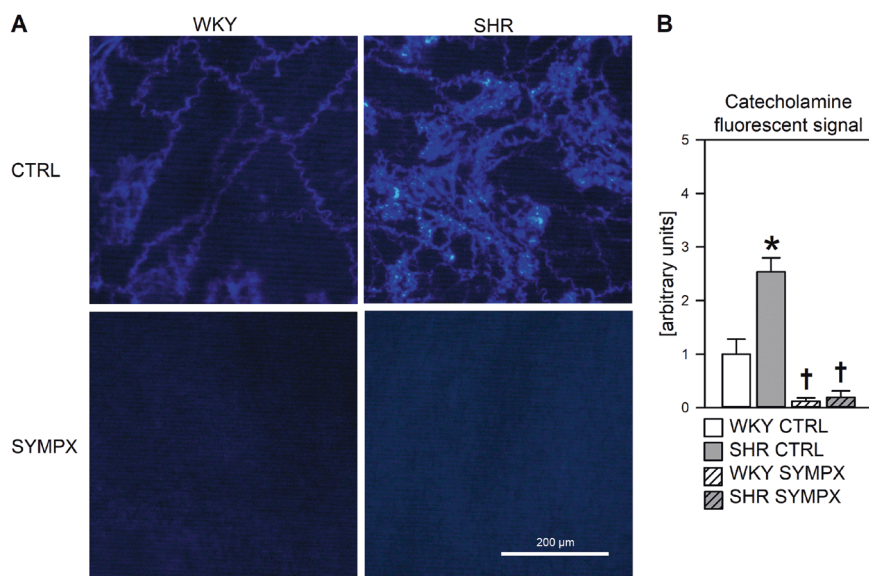
Spontaneous baroreflex function was determined in freely moving SHRs and WKY rats. SHRs had a reduced baroreflex sensitivity compared to that of WKY rats during both the light and dark phases (Table 2). The number of spontaneous baroreflex sequences was substantially decreased by sympathectomy, and baroreflex sensitivity during the dark phase was moderately but significantly increased in SYMPX animals compared to controls.

Since 14-day guanethidine treatment had different effects on BP and LF SBPV in freely moving animals, we examined the responses of the MAP, HR and the LF SBPV to restraint stress, i.e., under the conditions when SNS is activated. We observed a higher MAP response to stress in SHRs than in WKY rats, and this response was attenuated by 14-day guanethidine treatment (CTRL SHRs,  $29 \pm 2$  mmHg; CTRL WKY rats,  $14 \pm 2$  mmHg; SYMPX SHRs,  $7 \pm 3$  mmHg; SYMPX WKY rats,  $0 \pm 2$  mmHg; strain:  $F_{1,12} = 24.4$ ,  $p < 0.001$ ; treatment:  $F_{1,12} = 42.4$ ,  $p < 0.001$ ; interaction:  $F_{1,12} = 2.7$ , NS). The stress-induced HR increase, which was similar in both strains, was attenuated by sympathectomy (CTRL SHRs,  $109 \pm 12$  bpm; CTRL WKY rats,  $102 \pm 13$  bpm; SYMPX SHRs,  $23 \pm 6$  bpm; SYMPX WKY rats,  $31 \pm 4$  bpm; strain:  $F_{1,12} = 0.03$ , NS; treatment:  $F_{1,12} = 74.9$   $p < 0.001$ ; interaction:  $F_{1,12} = 0.7$ , NS). The response of the LF SBPV to stress was markedly higher in CTRL SHRs than in WKY rats, and sympathectomy decreased this parameter in both strains (CTRL SHRs,  $19.3 \pm 2.2$  mmHg<sup>2</sup>; CTRL WKY rats,  $5.9 \pm 1.2$  mmHg<sup>2</sup>; SYMPX SHRs,  $2.7 \pm 0.6$  mmHg<sup>2</sup>; SYMPX WKY rats,  $2.2 \pm 0.9$  mmHg<sup>2</sup>; interaction between strain and treatment:  $F_{1,12} = 18.9$ ,  $p < 0.001$ ). Thus, after 14 days of guanethidine treatment, sympathectomy-induced cardiovascular changes (decrease in the MAP, HR and the LF SBPV) were more evident under stressful conditions than in freely moving animals.

**Table 2** Low- and high-frequency domain power of systolic blood pressure variability (LF SBPV, HF SBPV), spontaneous baroreflex sensitivity (sBRS) and number of baroreflex events (BRR) in control (CTRL) and sympathectomized (SYMPX) WKY and SHR rats

	LF SBPV (mmHg <sup>2</sup> )		HF SBPV (mmHg <sup>2</sup> )		sBRS (ms/mmHg)		BRR sequences (n)	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark
<b>WKY</b>								
CTRL D0	3.7 ± 0.3	4.8 ± 0.5	2.4 ± 0.1	2.4 ± 0.2	2.2 ± 0.2	2.1 ± 0.1	389 ± 94	480 ± 94
SYMPX D3	0.9 ± 0.1	1.1 ± 0.1 <sup>†</sup>	1.9 ± 0.1	1.9 ± 0.1	2.3 ± 0.3	2.6 ± 0.3	36 ± 3	96 ± 26 <sup>†</sup>
SYMPX D14	0.9 ± 0.1	0.9 ± 0.1 <sup>†</sup>	1.7 ± 0.1	1.6 ± 0.1	2.4 ± 0.3	2.5 ± 0.4	77 ± 16	109 ± 21 <sup>†</sup>
<b>SHR</b>								
CTRL D0	4.6 ± 0.4	8.3 ± 0.6*	3.1 ± 0.1	3.0 ± 0.1	1.9 ± 0.1	1.6 ± 0.1	272 ± 17	319 ± 30*
SYMPX D3	2.0 ± 0.2	2.4 ± 0.1* <sup>†</sup>	2.6 ± 0.2	2.6 ± 0.1	1.8 ± 0.1	1.8 ± 0.1	72 ± 11	126 ± 14 <sup>†</sup>
SYMPX D14	2.1 ± 0.2	3.1 ± 0.3* <sup>†</sup>	2.7 ± 0.1	2.6 ± 0.1	1.8 ± 0.1	1.8 ± 0.1	115 ± 12	200 ± 27
<b>Factor</b>								
Strain	F <sub>1,22</sub> = 14.2 <i>p</i> < 0.01	F <sub>1,22</sub> = 38.0 <i>p</i> < 0.001	F <sub>1,22</sub> = 32.6 <i>p</i> < 0.001	F <sub>1,22</sub> = 45.0 <i>p</i> < 0.001	F <sub>1,24</sub> = 7.9 <i>p</i> < 0.05	F <sub>1,24</sub> = 17.9 <i>p</i> < 0.001	F <sub>1,24</sub> = 0.2 NS	F <sub>1,24</sub> = 0.1 NS
Treatment	F <sub>2,22</sub> = 114 <i>p</i> < 0.001	F <sub>2,22</sub> = 155 <i>p</i> < 0.001	F <sub>2,22</sub> = 6.4 <i>p</i> < 0.001	F <sub>2,22</sub> = 26.6 <i>p</i> < 0.001	F <sub>2,24</sub> = 0.1 NS	F <sub>2,24</sub> = 6.2 <i>p</i> < 0.01	F <sub>2,24</sub> = 31.3 <i>p</i> < 0.001	F <sub>2,24</sub> = 34.4 <i>p</i> < 0.001
Interaction	F <sub>2,22</sub> = 0.4 NS	F <sub>2,22</sub> = 6.4 <i>p</i> < 0.01	F <sub>2,22</sub> = 0.8 NS	F <sub>2,22</sub> = 3.1 NS	F <sub>2,24</sub> = 0.1 NS	F <sub>2,24</sub> = 1.4 NS	F <sub>2,24</sub> = 2.8 NS	F <sub>2,24</sub> = 6.1 <i>p</i> < 0.01

Cardiovascular parameters were measured by radiotelemetry in freely-moving animals during the light and dark phase. Data are the means ± SEM of 1 day before guanethidine treatment (D0), the third day of guanethidine treatment (D3) and the 14th day of guanethidine treatment (D14). *n* = 6–7 for each group. Statistical significance was computed by repeated measures two-way ANOVA, in the case of interaction Bonferroni post-hoc test was performed; \**p* < 0.05 vs WKY rats; <sup>†</sup>*p* < 0.05 vs CTRL rats (D0) of the same strain.



**Fig. 1** Histochemical visualization of catecholamines in the femoral arteries (SPG method). Representative images of control (CTRL) and sympathectomized (SYMPX) WKY rats and SHRs (**a**) and the quantitative evaluation of the fluorescent signal (**b**). The values are expressed as the mean ± SEM relative to CTRL WKY rats; *n* = 8–9

rats in each group. The effects of strain and treatment and their interaction were analyzed by two-way ANOVA (the results are shown in the text), and in the case of a significant interaction between the factors, Bonferroni post-hoc test was performed. \**p* < 0.05 vs. WKY rats; <sup>†</sup>*p* < 0.05 vs. CTRL rats

## Cardiovascular response to vasoactive agents

Responses to vasoactive agents were measured in conscious cannulated CTRL and SYMPX SHR and WKY rats to obtain more detailed information about the effects of sympathectomy on the cardiovascular system. The basal MAP (measured in rats placed in small plastic cages) was higher in SHRs than in WKY rats, and sympathectomy decreased the basal MAP (CTRL SHRs,  $157 \pm 2$  mmHg; CTRL WKY rats,  $102 \pm 2$  mmHg; SYMPX SHR,  $136 \pm 2$  mmHg; SYMPX WKY rats,  $91 \pm 3$  mmHg; strain:  $F_{1,66} = 233.9$ ,  $p < 0.001$ ; treatment:  $F_{1,66} = 16.7$ ,  $p < 0.001$ ; interaction:  $F_{1,66} = 3.9$ ,  $p = 0.053$ ). Basal HR was higher in SHRs than in WKY rats, and HR was decreased by sympathectomy (CTRL SHRs,  $363 \pm 1$  bpm; CTRL WKY rats,  $323 \pm 8$  bpm; SYMPX SHRs,  $318 \pm 6$  bpm; SYMPX WKY rats,  $282 \pm 10$  bpm; strain:  $F_{1,65} = 44.1$ ,  $p < 0.001$ ; treatment:  $F_{1,65} = 58.8$ ,  $p < 0.001$ ; interaction:  $F_{1,65} = 2.0$ , NS).

The ganglionic blocker pentolinium decreased the MAP in all experimental groups (CTRL SHRs,  $-47 \pm 3$  mmHg; CTRL WKY rats,  $-38 \pm 1$  mmHg; SYMPX SHRs,  $-7 \pm 4$  mmHg; SYMPX WKY rats,  $-15 \pm 4$  mmHg; interaction between strain and treatment:  $F_{1,26} = 8.1$ ,  $p < 0.01$ ). The effect of pentolinium on the MAP was more pronounced in CTRL SHRs than in WKY rats ( $p < 0.05$ ), and this strain difference was abolished by sympathectomy. Pentolinium decreased the HR more in CTRL WKY rats than in SHRs, and guanethidine treatment altered the HR response to pentolinium in both strains (CTRL SHRs,  $-16 \pm 5$  bpm; CTRL WKY rats,  $-26 \pm 6$  bpm; SYMPX SHRs,  $26 \pm 6$  bpm; SYMPX WKY rats,  $-10 \pm 10$  bpm; strain:  $F_{1,25} = 11.2$ ,  $p < 0.01$ ; treatment:  $F_{1,25} = 17.7$ ,  $p < 0.001$ ; interaction:  $F_{1,25} = 3.4$ , NS). The catecholamine-releasing agent tyramine increased the MAP similarly in SHRs and WKY rats, and the effect was almost completely abolished by sympathectomy (CTRL SHRs,  $48 \pm 4$  mmHg; CTRL WKY rats,  $49 \pm 5$  mmHg; SYMPX SHRs,  $4 \pm 1$  mmHg; SYMPX WKY rats,  $6 \pm 2$  mmHg; strain:  $F_{1,65} = 0.005$ , NS; treatment:  $F_{1,65} = 215.8$ ,  $p < 0.001$ ; interaction:  $F_{1,65} = 1.7$ , NS). The effect of tyramine on HR was similar to its effect on the MAP (CTRL SHRs,  $93 \pm 13$  bpm; CTRL WKY rats,  $94 \pm 15$  bpm; SYMPX SHRs,  $-3 \pm 3$  bpm; SYMPX WKY rats,  $2 \pm 2$  bpm; strain:  $F_{1,65} = 0.2$ , NS; treatment:  $F_{1,65} = 94.7$ ,  $p < 0.001$ ; interaction:  $F_{1,65} = 1.1$ , NS). The experiments with pentolinium and tyramine confirmed the persistent effect of sympathectomy after 14 days of guanethidine administration.

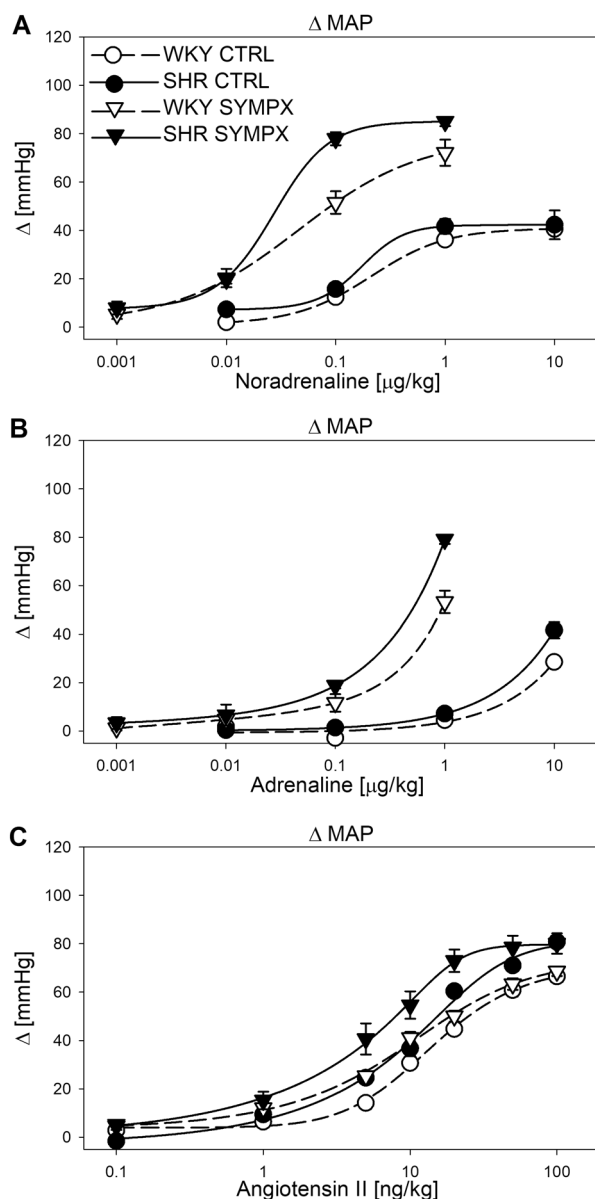
The acute administration of the angiotensin-converting enzyme inhibitor captopril decreased the MAP more in SHRs than in WKY rats, and sympathectomy did not change the MAP response to captopril (CTRL SHRs,  $-15 \pm 4$  mmHg; CTRL WKY rats,  $-4 \pm 1$  mmHg; SYMPX SHRs,  $-18 \pm 3$  mmHg; SYMPX WKY rats,  $-10 \pm 2$  mmHg; strain:  $F_{1,36} = 8.6$ ,  $p < 0.01$ ; treatment:  $F_{1,36} = 2.3$ , NS; interaction:

$F_{1,36} = 0.2$ , NS). Captopril administration caused an increase in HR in WKY rats but not in SHRs with or without sympathectomy (CTRL SHRs,  $2 \pm 7$  bpm; CTRL WKY rats,  $29 \pm 7$  bpm; SYMPX SHRs,  $7 \pm 5$  bpm; SYMPX WKY rats,  $32 \pm 8$  bpm; strain:  $F_{1,36} = 16.0$ ,  $p < 0.01$ ; treatment:  $F_{1,36} = 0.4$ , NS; interaction:  $F_{1,36} = 0.001$ , NS).

BP changes upon the administration of increasing doses of noradrenaline, adrenaline, and angiotensin II were analyzed by three-way ANOVA with the following factors: strain (SHRs vs. WKY rats), treatment (CTRL rats vs. SYMPX rats), and the dose of the particular vasoconstrictor. Regarding the MAP response to noradrenaline administration, the effect of strain vs. treatment interaction on the MAP response depended on the noradrenaline dose (Fig. 2a; strain vs. treatment interaction:  $p < 0.001$  at  $0.01 \mu\text{g/kg}$  dose, NS at  $0.1 \mu\text{g/kg}$  dose,  $p < 0.001$  at  $1 \mu\text{g/kg}$  dose). There was no strain difference in the increase in the MAP after  $0.01 \mu\text{g/kg}$  noradrenaline administration, and sympathectomy augmented the MAP response in both strains ( $p < 0.001$  for WKY rats,  $p < 0.01$  for SHRs). Upon the administration of noradrenaline at a dose of  $0.1 \mu\text{g/kg}$ , the increase in the MAP was more pronounced in SHRs than that in WKY rats ( $p < 0.001$ ), and sympathectomy augmented the MAP response in both strains ( $p < 0.001$ ). Upon the administration of noradrenaline at a dose of  $1 \mu\text{g/kg}$ , the MAP responses in CTRL SHRs and WKY rats were similar, sympathectomy augmented the responses in both strains ( $p < 0.001$ ), and SYMPX SHRs responded with a greater increase in the MAP than that in SYMPX WKY rats ( $p < 0.001$ ). When the MAP response to adrenaline administration was analyzed by three-way ANOVA, there was no significant interaction between strain, treatment, and dose. The MAP response to adrenaline was augmented in SHRs regardless of the adrenaline dose or guanethidine treatment (Fig. 2b). The effect of sympathectomy on the MAP response depended on the adrenaline dose. The MAP response upon the administration of  $0.01 \mu\text{g/kg}$  adrenaline was similar in CTRL and SYMPX rats, but the MAP responses were augmented in SYMPX rats upon the administration of  $0.1$  and  $1 \mu\text{g/kg}$  adrenaline ( $p < 0.001$  for both doses). When the MAP response to Ang II administration was analyzed by three-way ANOVA, there was no significant interaction between strain, treatment, and dose (Fig. 2c). The effect of both strain and treatment on the MAP response to Ang II depended on the dose. The MAP responses were more pronounced in SHRs than in WKY rats upon the administration of  $5$ ,  $10$ ,  $20$ ,  $50$ , and  $100 \text{ ng/kg}$  Ang II ( $p < 0.01$ ). Sympathectomy augmented the MAP responses upon the administration of  $5$ ,  $10$ , and  $20 \mu\text{g/kg}$  Ang II ( $p < 0.01$ ).

## Gene expression in vascular smooth muscle cells and in the adrenal medulla

The most abundantly expressed adrenergic receptors subtype in aortic smooth muscles was *Adra1d* followed by



**Fig. 2** Mean arterial pressure (MAP) response to the intravenous administration of vasoconstrictors. Noncumulative doses of noradrenaline (a), adrenaline (b), or angiotensin II (c) were administered to conscious control (CTRL) and sympathectomized (SYMPX) WKY rats and SHRs. The values are expressed as the mean  $\pm$  SEM;  $n = 6$ –8 rats for noradrenaline and adrenaline,  $n = 9$ –11 rats for angiotensin II. The effects of strain, treatment, and dose and their interactions were analyzed by three-way ANOVA (the results are listed in Supplementary Table 2), and in the case of significant interaction of the factors, Bonferroni post-hoc test was performed

*Adra1b* (Table 3). The mRNA expression of *Adra1a*, *Adra2a*, *Adra2b*, *Adra2c*, *Adrb1*, and *Adrb2* was too low for reliable quantification by quantitative real-time PCR. The mRNA expression levels of *Adra1d* and *Adra1b* were lower in SHRs than in WKY rats and were not affected by sympathectomy. The G-proteins that participate in  $\alpha_1$ -adrenergic contraction are the  $G_q$  and  $G_{11}$  subtypes,

which are encoded by the *Gnaq* and *Gna11* genes. The mRNA expression of *Gnaq* was lower in the vascular smooth muscles of SHRs than of WKY rats while the expression of *Gna11* was similar between the groups. There was no effect of sympathectomy on the mRNA expression of *Gnaq* or *Gna11*.

SHRs exhibited lower mRNA expression of enzymes involved in catecholamine biosynthesis (*Th*, *Ddc*, *Dbh*, and *Pnmt*) in the adrenal medulla than that exhibited by WKY rats (Table 3). There were no strain differences in the mRNA expression of genes involved in catecholamine reuptake or degradation (*Net*, *Comt*, *Maoa*, and *Maob*). Sympathectomy increased the mRNA expression of *Th*, *Dbh*, *Pnmt*, and *Maob* in the adrenal medulla.

### Catecholamine content in the adrenal gland

There was a lower noradrenaline content in the adrenal glands of CTRL SHRs than of WKY rats, and sympathectomy increased the noradrenaline content in the adrenal glands of WKY rats (CTRL SHRs,  $2.4 \pm 0.3 \mu\text{g}$ ; CTRL WKY rats,  $4.8 \pm 0.3 \mu\text{g}$ ; SYMPX SHRs,  $2.6 \pm 0.2 \mu\text{g}$ ; SYMPX WKY rats,  $7.3 \pm 0.8 \mu\text{g}$  per adrenal gland; interaction between strain and treatment:  $F_{1,27} = 4.5$ ,  $p < 0.05$ ). SHRs also exhibited a lower adrenal content of adrenaline than that of WKY rats, and sympathectomy increased the adrenaline content in the adrenal glands irrespective of strain (CTRL SHRs,  $12.8 \pm 0.4 \mu\text{g}$ ; CTRL WKY rats,  $14.0 \pm 0.5 \mu\text{g}$ ; SYMPX SHRs,  $13.4 \pm 0.4 \mu\text{g}$ ; SYMPX WKY rats,  $16.1 \pm 0.6 \mu\text{g}$  per adrenal gland; strain:  $F_{1,27} = 13.1$ ,  $p < 0.001$ ; treatment:  $F_{1,27} = 6.3$ ,  $p < 0.05$ ; interaction:  $F_{1,27} = 1.8$ , NS).

### Plasma catecholamines and corticosteroids

There was no strain difference in the plasma levels of noradrenaline or normetadrenaline in CTRL rats. Sympathectomy decreased the plasma levels of noradrenaline and normetadrenaline in both strains (Fig. 3a, b). CTRL SHRs exhibited similar plasma adrenaline levels but higher plasma metadrenaline levels compared to those exhibited by CTRL WKY rats (Fig. 3c, d;  $p < 0.05$ ). Sympathectomy increased the plasma levels of adrenaline and metadrenaline, and this effect was more pronounced in WKY rats (fourfold increase in SYMPX WKY rats,  $p < 0.001$  vs. 1.5-fold increase in SYMPX SHRs,  $p < 0.001$  for WKY rats, and  $p < 0.05$  for SHRs). The evaluation of plasma corticosteroid levels revealed that CTRL SHRs exhibited plasma levels of corticosterone similar to those of WKY rats (Fig. 3e) and plasma aldosterone levels lower than those of WKY rats (Fig. 3f,  $p < 0.01$ ). Sympathectomy increased the plasma levels of both corticosterone and aldosterone in WKY rats ( $p < 0.05$  for corticosterone and  $p < 0.001$  for aldosterone) but not in SHRs.

**Table 3** The mRNA expression of genes for adrenergic receptors and G-proteins in vascular smooth muscle cells of aorta and genes of catecholaminergic system in adrenal medulla

Tissue	Genes symbol	Ratio mean SHR/mean WKY (arbitrary units)				Two-way ANOVA		
		CTRL		SYMPX		Strain	Treatment	Interaction
		WKY	SHR	WKY	SHR			
Vascular smooth muscle cells	<i>Adra1d</i>	1.01 ± 0.05	0.72 ± 0.05	1.05 ± 0.08	0.75 ± 0.03	$F_{1,28} = 27.8$ $p < 0.001$	$F_{1,28} = 0.6$ NS	$F_{1,28} = 0.9$ NS
	<i>Adra1b</i>	1.04 ± 0.10	0.87 ± 0.06	1.08 ± 0.12	0.93 ± 0.04	$F_{1,28} = 4.2$ $p = 0.051$	$F_{1,28} = 0.6$ NS	$F_{1,28} = 0.3$ NS
	<i>Gnaq</i>	1.03 ± 0.1	0.92 ± 0.05	1.08 ± 0.06	0.95 ± 0.04	$F_{1,28} = 4.4$ $p < 0.05$	$F_{1,28} = 0.6$ NS	$F_{1,28} = 1.1$ NS
	<i>Gnal1</i>	1.02 ± 0.07	0.95 ± 0.05	0.92 ± 0.07	1.01 ± 0.03	$F_{1,28} = 0.02$ NS	$F_{1,28} = 0.1$ NS	$F_{1,28} = 0.2$ NS
Adrenal medulla	<i>Th</i>	1.01 ± 0.05	0.91 ± 0.09	1.43 ± 0.16	1.10 ± 0.05	$F_{1,28} = 4.7$ $p < 0.05$	$F_{1,28} = 9.8$ $p < 0.01$	$F_{1,28} = 1.3$ NS
	<i>Ddc</i>	1.01 ± 0.05	0.77 ± 0.04	0.99 ± 0.07	0.81 ± 0.04	$F_{1,28} = 15.5$ $p < 0.001$	$F_{1,28} = 0.1$ NS	$F_{1,28} = 0.4$ NS
	<i>Dbh</i>	1.02 ± 0.09	0.33 ± 0.05	1.5 ± 0.25	0.46 ± 0.04	$F_{1,28} = 42.1$ $p < 0.001$	$F_{1,28} = 5.2$ $p < 0.05$	$F_{1,28} = 1.7$ NS
	<i>Pnmt</i>	1.01 ± 0.05	0.65 ± 0.07	1.32 ± 0.08	0.98 ± 0.07	$F_{1,28} = 18.3$ $p < 0.001$	$F_{1,28} = 17.2$ $p < 0.001$	$F_{1,28} = 0.1$ NS
	<i>Net</i>	1.07 ± 0.14	1.05 ± 0.15	0.74 ± 0.12	0.93 ± 0.06	$F_{1,26} = 0.5$ NS	$F_{1,26} = 3.0$ NS	$F_{1,26} = 0.7$ NS
	<i>Comt</i>	1.01 ± 0.06	1.05 ± 0.04	1.13 ± 0.06	1.14 ± 0.06	$F_{1,28} = 0.2$ NS	$F_{1,28} = 3.2$ NS	$F_{1,28} = 0.1$ NS
	<i>Maoa</i>	1.01 ± 0.05	1.09 ± 0.05	0.99 ± 0.07	1.04 ± 0.06	$F_{1,27} = 1.3$ NS	$F_{1,27} = 0.4$ NS	$F_{1,27} = 0.1$ NS
	<i>Maob</i>	1.01 ± 0.07	0.91 ± 0.11	1.06 ± 0.09	1.25 ± 0.08	$F_{1,28} = 0.3$ NS	$F_{1,28} = 4.6$ $p < 0.05$	$F_{1,28} = 2.6$ NS

The values are expressed relatively to control (CTRL) WKY rats as the mean ± SEM,  $n = 7-8$  for each group. Statistical significance was computed by two-way ANOVA

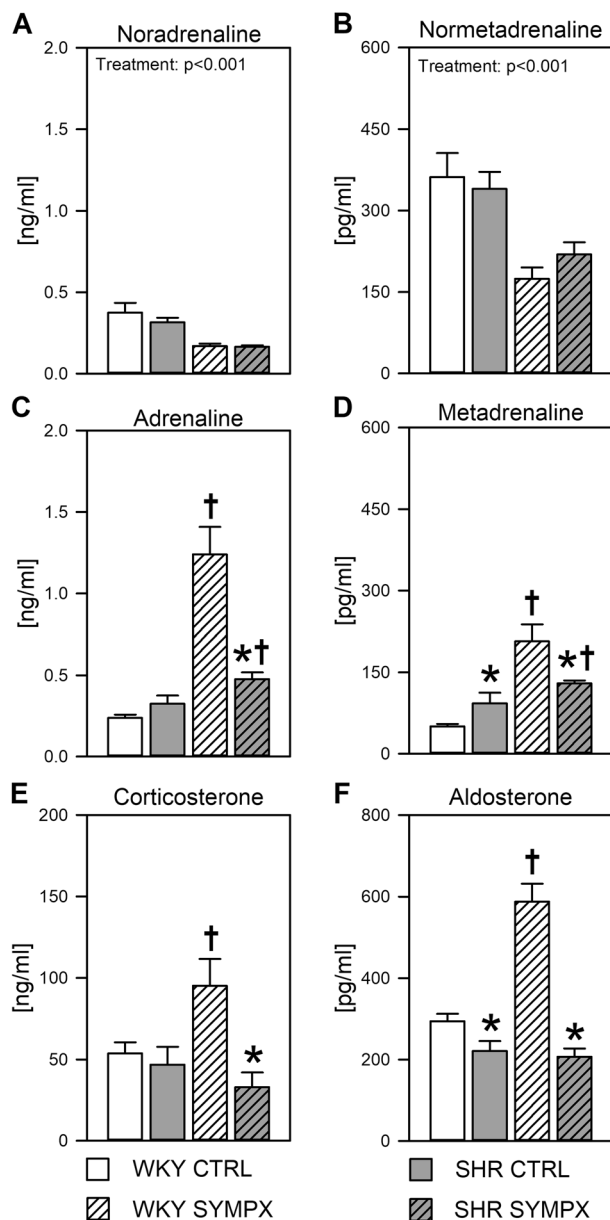
## Discussion

In the present study, the effects of guanethidine-induced sympathectomy on the cardiovascular system and adrenal glands of adult hypertensive SHR and normotensive WKY rats were evaluated. We demonstrated that chronic guanethidine treatment in SHR and WKY rats decreased the MAP, HR, LF SBPV, and HF SBPV (markers of sympathetic vascular and cardiac activity, respectively [26]). However, the MAP decrease was only temporary. During the light phase, the MAP returned to basal levels in both strains after 14-day guanethidine treatment despite the persistent effect of sympathectomy (as demonstrated by several measurements, i.e., decreased LF SBPV, reduced MAP response to pentolinium and tyramine, and low catecholamine content in the vascular wall). Moreover, stress-induced MAP and HR increases were attenuated by sympathectomy in both strains. MAP recovery in sympathectomized animals may be explained by compensatory mechanisms, e.g., the augmented BP response to catecholamines and/or increased plasma adrenaline levels. Plasma

adrenaline levels increased more in sympathectomized WKY rats than in SHRs, and this increase was accompanied by higher plasma levels of other adrenal hormones, such as corticosterone and aldosterone, in WKY rats only.

Our study confirmed the greater role of the SNS in SHRs than in WKY rats, as documented by higher LF SBPV and HF SBPV, a more pronounced MAP decrease after treatment with the ganglionic blocker pentolinium and a higher fluorescent signal of catecholamines in the femoral arteries in SHRs, which is in accordance with previous reports [4, 15, 23, 27, 28]. Sympathectomy reduced the MAP, HR, and the LF SBPV and HF SBPV in both SHRs and WKY rats after 3 days of guanethidine treatment. HR and the LF and HF SBPV were still reduced after 14 days of guanethidine treatment, but the MAP returned back to the control level after this period. Accordingly, it was reported that chronic guanethidine treatment of adult Sprague-Dawley rats either decreased [29] or did not significantly change [13] the resting BP. However, a decrease in the MAP after 14 days of guanethidine administration was observed under the conditions of restraint stress, as we





**Fig. 3** Plasma concentrations of adrenal hormones and their metabolites. Noradrenaline (a), adrenaline (b), normetadrenaline (c), metadrenaline (d), corticosterone (e), and aldosterone (f) levels in control (CTRL) and sympathectomized (SYMPX) WKY rats and SHRs. The values are expressed as the mean  $\pm$  SEM;  $n = 8$  rats for each group. The effects of strain and treatment and their interaction were analyzed by two-way ANOVA (the results are listed in Supplementary Table 3), and in the case of a significant interaction between the factors, Bonferroni post-hoc test was performed. \* $p < 0.05$  vs. WKY rats; † $p < 0.05$  vs. CTRL rats

previously demonstrated [15]; thus, the method of BP measurement might influence the results of the experiment. MAP recovery in freely moving animals cannot be ascribed to an insufficient degree of sympathectomy in either rat strain. The LF SBPV was still decreased after 14 days of guanethidine treatment. In both SHRs and WKY rats, guanethidine treatment decreased the catecholamine

fluorescent signal to  $<15\%$  of that in nonsympathectomized controls. Moreover, the MAP responses to pentolinium and tyramine were also attenuated by sympathectomy.

However, after the disruption of sympathetic innervation to the vasculature, vascular tone can be maintained by the increased postjunctional sensitivity of vascular smooth muscle cells to various vasoconstrictors [18]. In our experiment, sympathectomy increased MAP sensitivity to noradrenaline (up to 14-fold) and adrenaline (up to tenfold) in both SHRs and WKY rats. Accordingly, the increased BP response to the  $\alpha_1$ -adrenergic agonist phenylephrine has been demonstrated in adult sympathectomized Wistar [30] and WKY rats [31]. To determine whether the sympathectomy-induced augmentation of the MAP response is generalized or specific for catecholamines, the MAP response to Ang II was also measured. Sympathectomy increased the sensitivity of MAP to Ang II (up to twofold) in both SHRs and WKY rats, but this change was less pronounced than in the case of catecholamines. A smaller impact of sympathectomy on cardiovascular sensitivity to Ang II than to catecholamines was also described by Rizzoni et al. [31]. Kamikihara et al. [30] reported that increased vascular sensitivity to phenylephrine in animals sympathectomized by reserpine is accompanied by increased mRNA expression of *Adrald* in the rat tail artery. In the present study, we found no effect of guanethidine treatment on the mRNA expression of adrenergic receptors and G-protein subtypes that mediate adrenergic vasoconstriction in aortic smooth muscles. The contradictory results might be caused by the use of different sympatholytic agents or the examination of various vascular beds. Nevertheless, the increased sensitivity of the cardiovascular system to catecholamines can be achieved by mechanisms other than the de novo synthesis of adrenergic receptors, e.g., by their increased affinity for noradrenaline [32]. We also cannot exclude the possibility of vascular remodeling, which was described previously in the denervated rat caudal artery [33].

Spontaneous baroreflex function was evaluated in freely moving SHRs and WKY rats. We demonstrated that SHRs had lower baroreflex sensitivity than that of WKY rats and that sympathectomy improved baroreflex sensitivity in both strains. This finding is in accordance with reports that impaired baroreflex sensitivity is associated with sympathetic hyperactivity in SHRs as well as in human essential hypertension [10, 34], and baroreflex sensitivity can be augmented by sympathectomy [10, 35]. However, strain differences in baroreflex sensitivity persisted in sympathectomized SHRs and WKY rats. Impaired baroreflex function in SHRs is consistent with the augmented MAP decrease, but the attenuated HR increase to the acute administration of the angiotensin-converting enzyme inhibitor captopril in SHRs when compared to WKY rats. On

the other hand, different HR responses to the ganglionic blocker pentolinium in SHR and WKY rats is probably not associated with baroreflex function (as pentolinium impairs the baroreflex response), but rather with a higher intrinsic rate of atrial pacemaker in SHR [36].

In our study, guanethidine treatment decreased the plasma levels of noradrenaline and normetadrenaline by 50% in both SHR and WKY rats, which is in line with the data obtained in neonatally sympathectomized SHR [37]. Sympathectomy also increased the plasma adrenaline and metadrenaline levels in both strains, with the effect being three times stronger in WKY rats. Plasma noradrenaline predominantly originates from peripheral sympathetic nerve endings, whereas the adrenal medulla is considered to be the source of <10% of plasma noradrenaline under normal conditions. However, the adrenal contribution to plasma noradrenaline levels can increase up to 30–45% in stressed rats [38], suggesting that the adrenal gland can partially compensate for the lack of noradrenaline. Adrenal hypertrophy, the increase in the adrenal levels of noradrenaline and adrenaline and a higher activity of adrenal *Th* have already been described in sympathectomized Sprague-Dawley rats [39, 40]. We also observed adrenal hypertrophy in guanethidine-treated rats, and this effect was more pronounced in WKY rats than in SHR. Adrenal growth was accompanied by an increase in adrenal adrenaline content in both strains and increased adrenal noradrenaline content in WKY rats. We previously reported lower mRNA expression of catecholamine biosynthetic enzymes in the adrenal glands of young and adult SHR when compared to WKY rats [4]. Sympathectomy of adult rats increased the mRNA expression of the *Th*, *Dbh*, and *Pnmt* genes similarly in the adrenal medulla of both SHR and WKY rats, and the strain difference persisted.

Finally, the plasma levels of corticosterone and aldosterone were increased twofold by sympathectomy in WKY rats but not in SHR. Aldosterone secretion is regulated by Ang II; thus, a greater contribution of the renin–angiotensin system to BP maintenance in sympathectomized animals might explain the increased plasma aldosterone levels. It has been shown that the sympathetic–renal interaction contributes to hypertension development in SHR [41], and renal denervation lowers BP in this strain [42]. Guanethidine decreases plasma renin activity while increasing Ang II receptors in the adrenal gland [39]. However, in our study, sympathectomy did not change the acute MAP response to angiotensin-converting enzyme inhibitor in either strain, and further experiments are necessary to determine the contribution of the kidney and renin–angiotensin system to BP maintenance in sympathectomized animals. On the other hand, both corticosterone and aldosterone are released under stressful conditions [43]. It is possible that sympathectomy is a more stressful intervention for WKY rats

than for SHR since body weight loss and adrenal growth were more pronounced in sympathectomized WKY rats than in sympathectomized SHR.

In conclusion, sympathectomy of adult hypertensive SHR and normotensive WKY rats by guanethidine administration decreased HR and improved baroreflex sensitivity in both strains. Basal BP was lowered by sympathectomy only temporarily, but the effect of guanethidine was revealed under stressful conditions. BP recovery might be explained by compensatory mechanisms, such as the more than tenfold increase in BP sensitivity to catecholamines and the increased plasma levels of adrenaline. The involvement of the renin–angiotensin system in BP maintenance in sympathectomized rats also cannot be excluded.

SHR are characterized by sympathetic hyperactivity and are often considered a model of human essential hypertension. Our data indicate that sympathectomy in adult animals with established hypertension transiently lowers BP. Nevertheless, the long-term reduction of sympathetic vascular tone (decreased LF SBPV) is associated with HR reduction, an attenuated BP response to stress and an enhancement of baroreflex sensitivity. These improvements might decrease overall cardiovascular risk represented by increased HR and low baroreflex sensitivity in hypertensive patients.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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