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S. CACANYIOVA[1], M. MAJZUNOVA[1,2], S. GOLAS[1], A. BERENYIOVA[1]

THE ROLE OF PERIVASCULAR ADIPOSE TISSUE AND ENDOGENOUS HYDROGEN SULFIDE IN VASOACTIVE RESPONSES OF ISOLATED MESENTERIC ARTERIES IN NORMOTENSIVE AND SPONTANEOUSLY HYPERTENSIVE RATS

[1]Institute of Normal and Pathological Physiology, Center of Experimental Medicine, Slovak Academy of Sciences, Bratislava, Slovak Republic; [2]Department of Animal Physiology and Ethology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovak Republic

Perivascular adipose tissue (PVAT) and hydrogen sulfide (H_2S) play important roles in the modulation of vasoactive responses and can interfere with the ethiopathogenesis of essential hypertension. The aim of this study was to evaluate the mutual relationship between PVAT and H_2S (endogenously produced, exogenous) in vasoactive responses of isolated mesenteric arteries (MA) in adult normotensive (Wistar) and spontaneously hypertensive rats (SHR). In SHR, hypertension was associated with cardiac hypertrophy and increased contractility; however, there were no differences in the amount of retroperitoneal fat between strains. PVAT revealed the anti-contractile effect on vasoconstriction induced by exogenous noradrenaline in both strains, but surprisingly, this effect was stronger in SHR. Concurrently; PVAT exhibited a pro-contractile effect on contractions to endogenous noradrenaline released from arterial sympathetic nerves in SHR, but not in Wistar rats. We confirmed the anti-contractile effect of H_2S in both, the vascular wall and PVAT of Wistar rats because the pre-treatment with propargylglycine (PPG), an inhibitor of H_2S producing enzyme, significantly increased the noradrenaline-induced contraction. In SHR, H_2S in the vascular wall exhibited a pro-contractile effect that was eliminated by the presence of PVAT; however, the pre-treatment with PPG did not affect noradrenaline contraction farther. Nevertheless, unlike in Wistar rats, the presence of PVAT potentiated the vasorelaxant effect of exogenously applied H_2S in SHR. Our results confirmed that PVAT of MA and endogenously produced H_2S could manifest as pro-contractile or as anti-contractile. In SHR, unlike in Wistar rats, the pro-contractile effect of PVAT associated with the stimulation of perivascular nerves, and the pro-contractile effect of H_2S in the arterial wall could represent pathologic features. On the other hand, PVAT of SHR is endowed with compensatory vasoactive mechanisms, which include stronger anti-contractile action of an unknown factor (other than H_2S) and potentiation of the vasorelaxant effect of exogenous H_2S.

Key words: *hydrogen sulfide, mesenteric artery, perivascular adipose tissue, Normotensive rats, spontaneously hypertensive rats, propargylglycine, nitric oxide, adipocyte-derived relaxing factor*

INTRODUCTION

Perivascular adipose tissue (PVAT) represents a specific deposit of adipose tissue surrounding blood vessels. In addition to providing mechanical protection, PVAT seems to be an important secretory organ due to its ability to release biologically effective molecules. The results obtained in recent years indicate that under physiological conditions, PVAT exerts predominantly anti-contractile effects, which are induced, beside others, by a transferable factor called adipocyte-derived relaxing factor (ADRF). Schleifenbaum *et al.* (1) proposed that ADRF could be hydrogen sulfide (H_2S), an important gaseous transmitter. They observed in mesenteric arteries and aortas of normotensive rats and mice that inhibition of endogenous H_2S had no effect on vessels without perivascular fat. However, inhibition of endogenous H_2S significantly reversed the anti-contractile effect of arteries with PVAT. Nevertheless, in addition to PVAT (2), H_2S can be produced by arterial smooth muscle cells (3) and endothelial cells (4). The presence of PVAT can also modulate the production of H_2S by arterial wall cells, *e.g.*, endothelial cells (5). Moreover, H_2S has been shown to have a dual vasoactive effect. In rat conduit arteries, vasoconstrictor and vasorelaxant effects have both been demonstrated (6). Whereas NO-independent pathways, predominantly K_{ATP} activation, are responsible for the vasorelaxing effects of H_2S, the vasoconstrictor effects of H_2S are likely related to downregulation of the L-arginine/NO pathway (3, 7). Nevertheless, contradictory results regarding the synergistic and antagonistic effects of H_2S and NO have been published. Coletta *et al.* (8) showed that NO and H_2S are mutually required for the physiological control of vascular function. The authors confirmed that pretreatment with H_2S donor potentiated the vasorelaxant response of the thoracic aorta to acetylcholine and to NO donor and increased cGMP levels. In addition, silencing

of a H_2S-producing enzyme, cystathionine-γ-lyase (CSE), resulted in a significant inhibition of the vasodilator responses of vascular rings to both vasodilators. Therefore, endogenously produced H_2S can operate in vascular tone control and blood pressure regulation in distinct ways.

Several papers confirmed that both systems, PVAT and H_2S, can interfere with the etiopathogenesis of essential hypertension. In spontaneously hypertensive rats (SHR), the beneficial effect of compounds produced by PVAT on the vascular wall could be reduced. Torok *et al.* (9) confirmed the anti-contractile effect of PVAT in mesenteric arteries from normotensive Wistar Kyoto rats. However, in SHR, the anti-contractile effect of PVAT was reduced. Similarly, Galvez *et al.* (10) found that SHR had less PVAT in the mesenteric bed and that the alterations in the visceral perivascular fat mass and function may contribute to the increased vascular resistance in this model of hypertension. Experiments using adult SHR, to which H_2S was administered, proved that H_2S partially prevented hypertension and arterial remodeling (6). Xiao *et al.* (11) showed that exogenous H_2S was able to inhibit reactive oxygen species production and to suppress vascular oxidative stress in hypertensive animals. Xue *et al.* (12) demonstrated that the protective effect of H_2S was attributable to, at least in part, suppression of vascular oxidative stress that involved inhibition of AT_1 receptor action, downregulation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, as well as upregulation of antioxidant enzyme. Zhao *et al.* (13) showed that NaHS could decrease the binding affinity of the AT_1 receptor in vascular smooth muscle cells of SHR so leading to decreased contractility. In our previous studies, we confirmed that exogenous H_2S could play a role in the compensatory mechanisms of SHR and counter-regulate the increased vascular tone to a significantly greater degree than in normotensive animals (14, 15). Nevertheless, the relationship between PVAT and H_2S endogenously produced by the arterial wall under conditions of essential hypertension has not been investigated to date. The aim of this study was to evaluate and compare the participation of PVAT endogenously produced H_2S in the contractile and relaxant responses as well as the effect of exogenous H_2S in isolated mesenteric arteries of normotensive Wistar rats and SHR.

MATERIALS AND METHODS

Experimental animals

Animals were bred in accordance with the institutional guidelines of the State Veterinary and Food Administration of the Slovak Republic and the Committee on the Ethics of Procedures in Animal, Clinical and others Biomedical Experiments (Permit Number: EC/CEM/2017/4) of the Centre of Experimental Medicine as well as in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes, Directive 2010/63/EU of the European Parliament. All rats used in this study were received from an accredited breeding establishment of the Institute of Normal and Pathological Physiology, Centre of Experimental Medicine, Slovak Academy of Sciences and were housed under a 12 h light-12 h dark cycle at a constant humidity (45 – 65%) and temperature (20 – 22°C) with free access to standard laboratory rat chow and drinking water (*ad libitum*).

Basic parameters

To examine the vasoactive properties of an isolated mesenteric artery, 16-week-old male Wistar rats (n = 8) and SHR (n = 8) were used in this study. The body weight (BW) of each rat was determined before decapitation. Systolic blood pressure (SBP) was measured in pre-warmed rats by non-invasive plethysmography of rat tail arteries before the beginning of the *in vitro* study. Rats were killed by decapitation after a brief anesthetization with CO_2, their heart and retroperitoneal fat were weighted and tibia length was measured. The superior mesenteric artery (MA) was isolated for the further *in vitro* functional examination. Plasma samples were collected just after the decapitation and frozen in aliquots for biochemical determinations. The basic levels of cholesterol (Chol), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), triacylglycerols (TAG) and glucose (GLU) were commercially determined in Laboklin GMBH. The values of parameters represented postprandial concentrations. We avoided 24-hour fasting because fasting stimulates lipid mobilization and lipolysis which could reduce mesenteric PVAT.

Drugs

The following drugs were used: propargylglycine, tyramine, sodium sulphide nonahydrate from Sigma-Aldrich (St Louis, Missouri, USA), and noradrenaline, from Zentiva (Prague, Czech Republic). All drugs were dissolved in distilled water.

Functional study

The vessels were divided into two groups, without PVAT ($PVAT^-$) and with intact PVAT ($PVAT^+$), to distinguish between the contribution of H_2S produced by arterial wall and the effect of total H_2S produced by the arterial wall and surrounding perivascular fat. Subsequently, the MA with intact endothelium was cleaned of connective tissue and cut into 3 mm length rings. Rings were mounted on thin wires and were horizontally fixed. Rings were immersed in a 10 mL incubation organ bath with Krebs solution (NaCl 118 mmol/L, KCl 5 mmol/L, $NaHCO_3$ 25 mmol/L, $MgSO_4.7H_2O$ 1.2 mmol/l; KH_2PO_4 1.2 mmol/L, $CaCl_2$ 2.5 mmol/L, glucose 11 mmol/L, $CaNa_2EDTA$ 0.032 mmol/L). This solution was oxygenated with a mixture of 95% O_2 and 5% CO_2 and kept at 37ºC. The changes in isometric tension were measured using electromechanical transducers (MDE, Budapest, Hungary) and registered using an AD converter and Dewetron software (Dewetron, Prague, Czech Republic). A resting tension of 0.7 g was applied to each ring and maintained throughout a 45- to 60-min equilibration period until stress relaxation no longer occurred.

KCl (125 mmol/L, physiological Krebs solution changed to a solution in which NaCl was exchanged for equimolar concentration of KCl) was added to the organ bath for only 2 min to confirm the sufficient contractility of the sample. After washing with physiological Krebs solution and an equilibration period, experiments with noradrenaline were started to obtain the contractile responses. Adrenergic contractions were determined in MA as the responses to cumulatively applied exogenous noradrenaline (NA, 0.0001 – 10 μmol/L) or as the neurogenic responses elicited by electrical stimulation of periarterial sympathetic nerves. For transmural nerve stimulation (TNS), arterial rings were mounted between two platinum electrodes placed on either side of the preparation and connected to an ST-3 electrostimulator (MDE, Hungary). Frequency-response curves to electrical stimuli were obtained using square pulses of 0.2 ms in duration at a supramaximal voltage of 35 V at 2 – 64 Hz for a period of 20 seconds. The TNS responses were pharmacologically tested in preliminary experiments. These contractions were abolished by tetrodotoxin, indicating the neurogenic nature of the responses. Guanethidine or phentolamine abolished the responses at all frequencies of stimulation. Therefore, the contractile responses were due to the

release of noradrenaline from depolarized perivascular nerves. Tyramine (1 – 1000 μmol/L), an indirect sympathomimetic drug, was used as a tool to test whether there was a functional pool of catecholamines (mainly NA) in PVAT.

To examine the participation of endogenously produced H_2S in the regulation of the basal tone, propargylglycine (PPG) (1 – 10 mmol/L) in cumulative concentrations was added to the organ bath. Although PPG could inhibit other enzymes in different tissues such as liver, heart, skeletal muscle, kidneys and brain, it is a commonly used inhibitor of cystathionine-γ-lyase (CSE), H_2S producing enzyme, in experiments with isolated arteries (16). To evaluate the participation of endogenous H_2S in vasoactive responses, the contractile responses induced by increasing concentrations of NA were followed before and 20 minutes after acute administration of PPG (10 mmol/L). Contractions were expressed in g as developed tension to demonstrate maximum reached contraction (E_{max}). To demonstrate the sensitivity of arterial wall all individual curves were expressed as a percentage of the maximum tissue response to NA (not shown) and the concentrations of NA producing the half-maximum response (EC_{50}) were calculated and expressed as the negative logarithm of NA molar concentration.

Na_2S was used to evaluate the vasoactive effect of exogenous H_2S. Na_2S dissociates in water solution to Na^+ and S^{2-}, which reacts with H^+ to yield HS^- and H_2S. We use the term Na_2S to encompass the total mixture of H_2S, HS^- and S^{2-}. Direct vasoactive effects of Na_2S were observed on NA-pre-contracted (1 μmol/L) rings by administration of increasing doses of Na_2S (20, 40, 80, 100, 200, 400 μmol/L). The rate of vasorelaxation was expressed as a percentage of the NA-induced contraction.

Statistical analysis

The data are expressed as the mean ± SEM. The effect of PVAT on the vasoactive responses of MA, NA-induced contraction, tyramine-induced contraction and TNS in Wistar and SHR were analyzed using two-way ANOVA (strain × PVAT) with Bonferroni *post-hoc* test. To evaluate general cardiovascular, plasmatic parameters one-way ANOVA was used with Bonferroni *post-hoc* test. Differences between means were considered significant at $P < 0.05$.

RESULTS

General characteristic of experimental animals

The comparison of the basic cardiovascular and functional parameters between Wistar (n = 8) rats and SHR (n = 8) is shown in *Table 1*. In SHR, a significant increase ($P < 0.01$) was observed in the SBP values. SHR had reduced body weight compared to Wistar rats ($P < 0.001$). On the other hand, the heart weight/body weight ratio and heart weight/tibia length were increased in this strain ($P < 0.01$ and $P < 0.05$). These results confirmed that the blood pressure increase demonstrated in SHR compared to normotensive rats was associated with cardiac hypertrophy, which is in a good agreement with original description of SHR strain.

There were no differences in the amount of retroperitoneal fat, glucose level and basic lipid profile determined in the plasma of Wistar rats and SHR with the exception of TAG that were significantly decreased in SHR compared to Wistar rats ($P < 0.01$).

Role of perivascular adipose tissue

Cumulative application of exogenous NA (0.0001 – 10 μmol/L) induced vasoconstriction in a concentration-dependent manner. PVAT had a significant effect on these responses ($F_{(1,207)} = 32.767$, $P = 3.614 \times 10^{-8}$). In Wistar rats (n = 8), the presence of PVAT attenuated NA-induced contractions only at a concentration of 0.1 μmol/L (proving the anti-contractile activity of PVAT), although the sensitivity of arterial wall to exogenous NA was significantly decreased after the PPG pre-treatment (*Table 3*). In SHR (n = 8), the reducing

Table 1. General characteristic of experimental animals.

Parameter	Wistar rats	Spontaneously hypertensive rats
SBP (mmHg)	128.14 ± 2.82	172.68 ± 2.22 **
BW (g)	424,5 ± 10.77	335.5 ± .73 ***
HW (mg)	1240 ± 28.81	1275 ± 26.47
TL (mm)	39.48 ± 0.51	37.16 ± 0,47
HW/BW (mg/g)	2.92 ± 0.05	3.80 ± 0.04 ***
HW/TL (mg/mm)	31.42 ± 0.66	34.17 ± 0.88 *
RFW (mg)	3320.38 ± 547.13	3060.25 ± 132.91
RFW/TL (mg/mm)	83.68 ± 12.98	81.29 ± 3.95
Chol (mg/dL)	2.05 ± 0.18	2.19 ± 0.10
HDL (mg/dL)	56.92 ± 5.44	64.98 ± 2.69
LDL (mg/dL)	10.33 ± 1.42	13.1 ± 0.56
TAG (mmol/L)	2.23 ± 0.13	1.39 ± 0.18 **
GLU (mmol/L)	7.48 ± 0.36	7.36 ± 0.41

Abbreviations: BW, body weight; Chol, total cholesterol; GLU, glucose; HDL, low-density lipoprotein cholesterol; HW, heart weight; HW/BW, heart weight/body weight ratio; HW/TL, heart weight/tibia length ratio; LDL, low-density lipoprotein cholesterol; RFW, retroperitoneal fat weight; RFW/TL, retroperitoneal fat weight/tibia length ratio; SBP, systolic blood pressure; TAG, triacylglycerols; TL, tibia length;
Values are mean ± S.E.M. *P < 0.05.; **P < 0.01;***P < 0.001 versus Wistar rats.

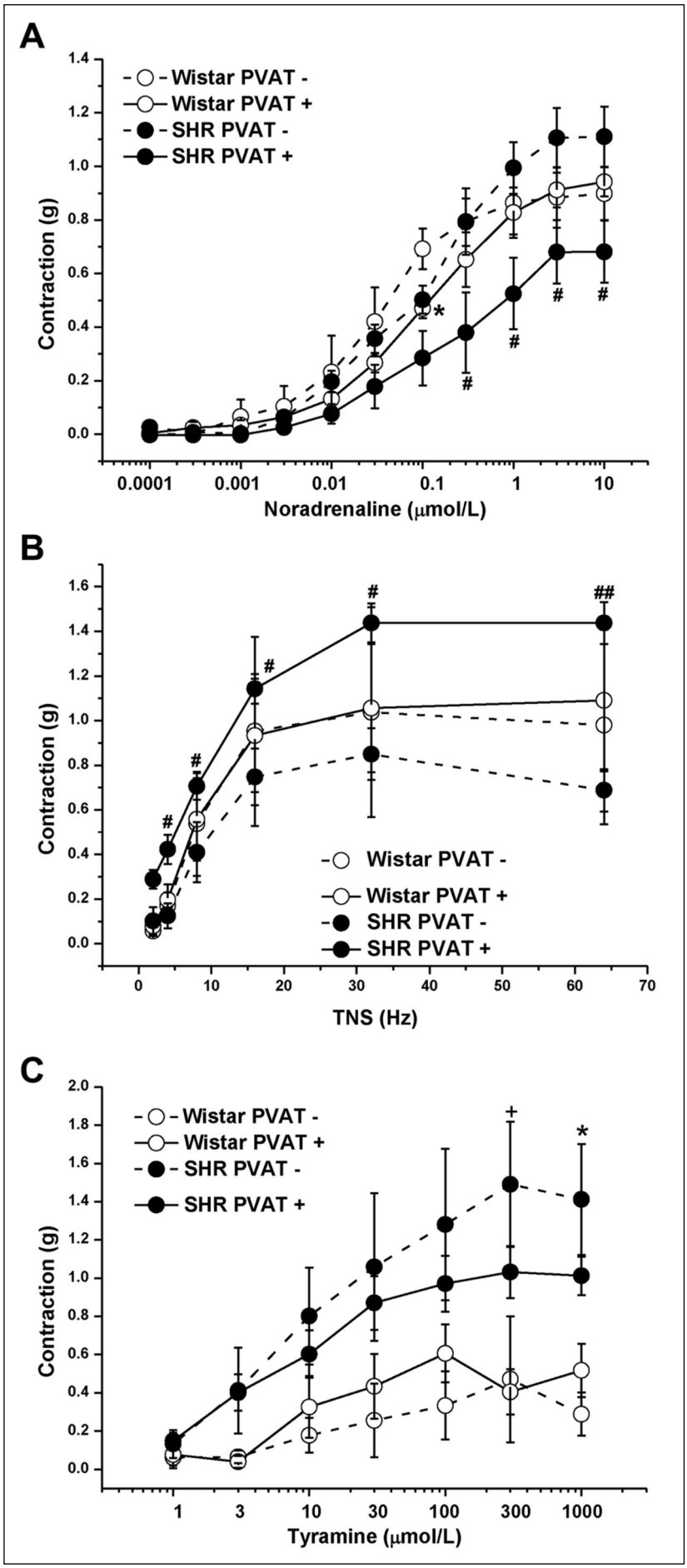

Fig. 1. The contractile responses of mesenteric arteries induced by exogenous noradrenaline (A), endogenous noradrenaline released from sympathetic nerves after transmural nerve stimulation (TNS) (B), and tyramine (C). Values are ± SEM. Wistar rats: n = 8; SHR: n = 8; *P < 0.05 versus Wistar $PVAT^{-}$; $^{+}P < 0.05$ versus Wistar $PVAT^{+}$; $^{\#}P < 0.05$; $^{\#\#}P < 0.01$ versus SHR $PVAT^{-}$.

effect of PVAT was recorded at a concentration of 0.3 – 10 µmol/L without significant changes in the sensitivity to NA (*Table 3*). Moreover, there was a significant effect of the strain ($F_{(1,207)}$ = 6.039, P = 0.015) and a strain-PVAT interaction ($F_{(1,207)}$ = 10.412, P = 0.0015) (*Fig. 1A*). Original traces of the vasoconstrictor responses induced by exogenous noradrenaline in PVAT intact and denuded MA are shown in *Fig. 2*.

Transmural nerve stimulation (TNS, 2 – 64 Hz) induced the release of endogenous NA from nerve endings in the arterial wall and evoked contractions of smooth muscle cells (SMC). However, in Wistar rats and SHR, the contractile responses were comparable between rings without (n = 8) and with (n = 8) PVAT (*Fig. 1B*). Generally, a significant effect of PVAT was shown in SHR ($F_{(1,84)}$ = 7.504, P = 0.008). In SHR, the potentiating effect was proved at a frequency of 4 Hz and 16 – 64 Hz.

Application of increasing concentrations of tyramine (1 – 1000 µmol/L) induced contractile responses in Wistar rats and SHR (*Fig. 1C*). PVAT had no significant effect on the responses, but there was a significant effect of the strain ($F_{(1,110)}$ = 46.264, P = 5.624×10^{-10}), and a strain-PVAT interaction ($F_{(1,110)}$ = 4.1024, P = 0.045) was also found. Moreover, no difference in TYRmax and EC50 values was observed (*Table 4*).

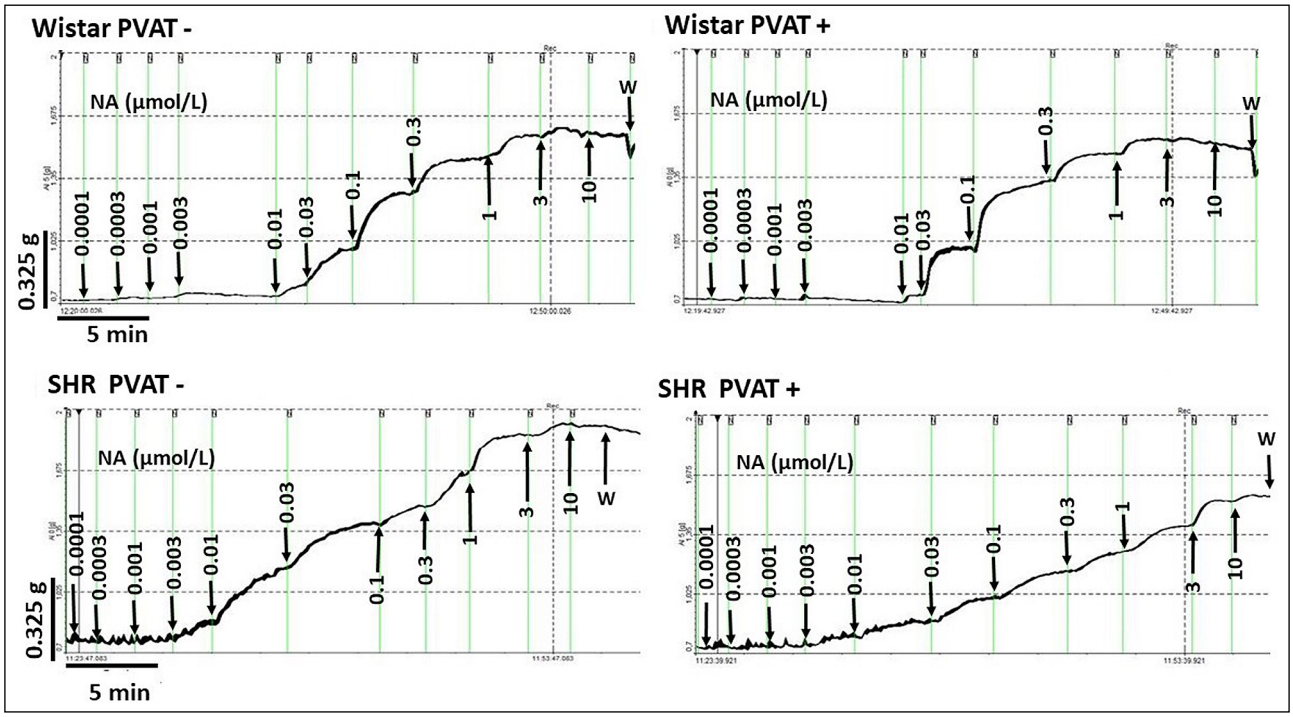

Fig. 2. Original record of the contractile responses induced by cumulative concentrations of exogenous noradrenaline (NA) in mesenteric arteries with intact ($PVAT^+$) or removed ($PVAT^-$) perivascular adipose tissue in Wistar rats and SHR. w = washout.

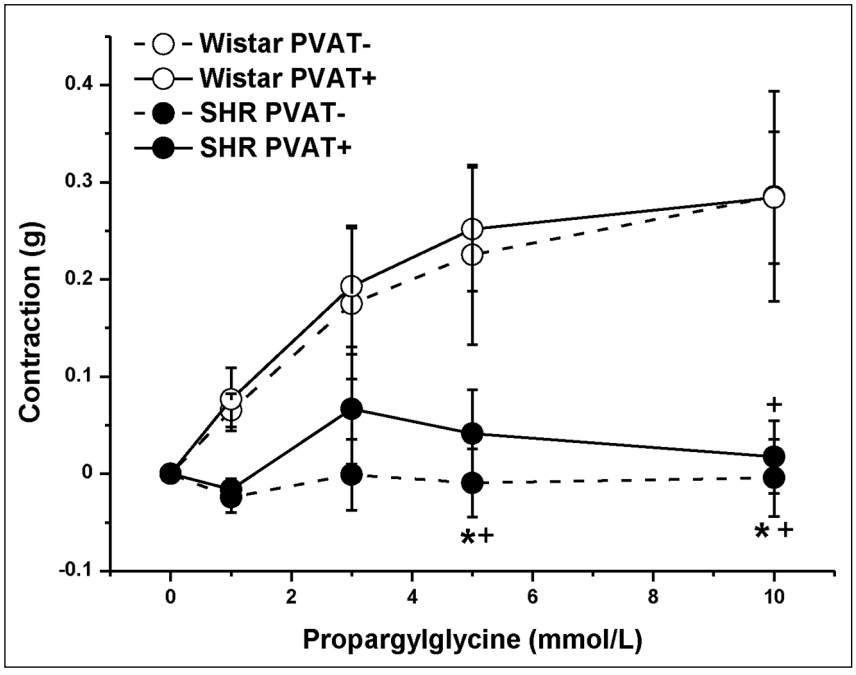

Fig. 3. Comparison of the dose-response curves to exogenous propargylglycine (PPG) in mesenteric arteries without PVAT and intact PVAT. Values are ± SEM. Wistar rats: n = 8; SHR: n = 8; *P < 0.05 versus Wistar $PVAT^-$; $^+$P < 0.05 versus Wistar $PVAT^+$.

Role of endogenous H_2S *inhibition*

The direct application of cumulative concentrations of a CSE inhibitor, PPG (1 – 10 mmol/L), evoked the opposite effect on the basal arterial tone in Wistar rats (n = 8) and SHR (n = 8). PPG application in Wistar rats had a vasocontractile character, contrary to SHR, in which nor (in PVAT denuded rings) or a slight decrease (in PVAT intact rings) in tension (5 – 10 mmol/L) was observed (*Fig. 3*). The vasoactive responses to PPG were significantly affected by the strain ($F_{(1,66)}$ = 48.71, P = 5.342 × 10^{-9}). The contractile responses observed in Wistar rats were reduced in SHR regardless of the PVAT presence. The differences in PPGmax and EC_{50} values are in *Table 5*.

The participation of endogenously produced H_2S in adrenergic contractions of MA was tested after acute pre-treatment with PPG (10 mmol/L, 20 min) in rings with intact or denuded PVAT. The changes of basal tension induced by the pre-treatment with PPG are shown in *Table 2*. In Wistar rats (n = 8), CSE

Table 2. The effect of pre-treatment with propargylglycine (10 mmol/L) on basal tension of mesenteric artery in Wistar and spontaneously hypertensive rats.

Propargylglycine (g)	**Wistar rats**		**Spontaneously hypertensive rats**	
	PVAT$^-$	PVAT$^+$	PVAT$^-$	PVAT$^+$
	0.33 ± 0.10	0.24 ± 0.10	–0.02 ± 0.03	–0.04 ± 0.02

PVAT, perivascular adipose tissue.
Positive values = increase in basal tension; negative values = decrease in basal tension.

Table 3. Characterization of noradrenaline-induced contractions in mesenteric arteries with perivascular adipose tissue removed (PVAT$^-$) and intact (PVAT$^+$) before and after the pre-treatment with propargylglycine, obtained from normotensive Wistar rats and spontaneously hypertensive rats.

	Wistar rats				**Spontaneously hypertensive rats**			
	PVAT$^-$		PVAT$^+$		PVAT$^-$		PVAT$^+$	
	PPG$^-$	PPG$^+$	PPG–	PPG+	PPG$^-$	PPG$^+$	PPG$^-$	PPG$^+$
NA_{max} (g)	0.8 ± 0.13	0.57 ± 0.17	0.89 ± 0.07	0.91 ± 0.08	1.11 ± 0.11	0.79 ± 0.18	0.63 ± 0.14$^{\#}$	0.56 ± 0.10$^{\#}$
EC_{50} (%)	8.36 ± 0.20	8.19 ± 0.27	7.22 ± 0.17**	7.61 ± 0.11	7.23 ± 0.18	6.81 ± 0.07$^{\#}$	6.91 ± 0.26	6.77 ± 0.08

EC_{50}, the negative logarithm of NA molar concentration inducing the half-maximum response; NA, noradrenaline; NA_{max}, maximum reached contraction; PPG, propargylglycine.
Values are mean ± S.E.M. **P < 0.01 versus Wistar PVAT$^-$ PPG$^-$; $^{\#}$P < 0.05 versus SHR PVAT$^-$ PPG$^-$

Table 4. Characterization of tyramine-induced contractions in mesenteric arteries with perivascular adipose tissue removed (PVAT$^-$) and intact (PVAT$^+$), obtained from normotensive Wistar and spontaneously hypertensive rats.

	Wistar rats		**Spontaneously hypertensive rats**	
	PVAT$^-$	PVAT$^+$	PVAT$^-$	PVAT$^+$
TYR_{max} (g)	0.29 ± 0.11	0.52 ± 0.14	1.49 ± 0.33$^+$	1.03 ± 0.13 *
EC_{50} (%)	4.78 ± 0.61	5.17 ± 0.19	5.39 ± 0.16	5.16 ± 0.15

EC_{50}, the negative logarithm of TYR molar concentration inducing the half-maximum response, TYR, tyramine; TYR_{max}, maximum reached contraction. Values are mean ± S.E.M, *P < 0.05 versus Wistar PVAT$^-$; $^+$P < 0.05 versus Wistar PVAT$^+$.

Table 5. Characterization of propargylglycine-induced contractions in mesenteric arteries with perivascular adipose tissue removed (PVAT–) and intact (PVAT+), obtained from normotensive Wistar and spontaneously hypertensive rats.

	Wistar rats		**Spontaneously hypertensive rats**	
	PVAT$^-$	PVAT$^+$	PVAT$^-$	PVAT$^+$
PPG_{max} (g)	0.29 ± 0.11	0.28 ± 0.07	–0.001 ± 0.04*$^+$	0.07 ± 0.06$^+$
EC_{50} (%)	2.86 ± 0.01	2.88 ± 0.04	2.94 ± 0.01	2.87 ± 0.04

EC_{50}, the negative logarithm of PPG molar concentration inducing the half-maximum response; PPG, propargylglycine; PPG_{max}, maximum reached contraction; EC_{50} = the negative logarithm of PPG molar concentration inducing the half-maximum response.
Values are mean ± S.E.M; *P < 0.05 versus Wistar PVAT$^-$; $^+$P < 0.05 versus Wistar PVAT$^+$

inhibition significantly increased exogenous NA-induced contractions in PVAT$^-$ rings (0.001 μmol/L; $P < 0.01$; 0.0003 μmol/L; $P < 0.05$), but the maximum force of the contractile responses was not changed (*Fig. 4*). Additionally, PPG pre-treatment did not affect the sensitivity of arterial wall to exogenous NA (*Table 3*). In PVAT$^+$ rings isolated from Wistar rats (n = 8), the acute PPG pre-treatment acted in a similar manner. PPG application significantly increased adrenergic contractions at doses 0.0001 – 0.01 μmol/L ($P < 0.05$) without affecting the maximal enriched contraction (*Fig. 4*). There was a significant effect of the PPG pre-treatment ($F_{(1,255)} = 10.89$, $P = 0.0011$) and a PVAT-PPG interaction ($F_{(1,255)} = 4.29$, $P = 0.0395$) was confirmed. No difference in EC_{50} values was observed (*Table 3*).

CSE inhibition evoked a reduction in the contractile responses in PVAT denuded rings (PVAT$^-$, n = 8) of SHR. The adrenergic contraction was significantly attenuated at a range of doses from 0.01 – 0.3 μmol/L ($P < 0.05$; $P < 0.01$), but the maximum force of the contractile responses was not changed (*Fig. 5*). The sensitivity of arterial wall to exogenous NA was significantly decreased after the PPG pre-treatment (*Table 3*). The presence of PVAT eliminated the effect of PPG application. No differences in absolute adrenergic contractions and the sensitivity of arterial wall to exogenous NA were shown in PVAT$^+$ rings of SHR (n = 8; *Table 3*, *Fig. 5*). The results generally confirmed an effect of PPG pre-treatment ($F_{(1,313)} = 28.11$, $P = 2.385 \times 10^{-7}$) and PVAT ($F_{(1,313)} = 50.76$, $P = 9.432 \times 10^{-12}$), moreover a PVAT × PPG interaction was observed ($F_{(1,313)} = 9.61$, $P = 0.002$). Original traces of the vasoconstrictor responses induced by exogenous noradrenaline before and after the treatment with PPG are shown in *Fig. 6*.

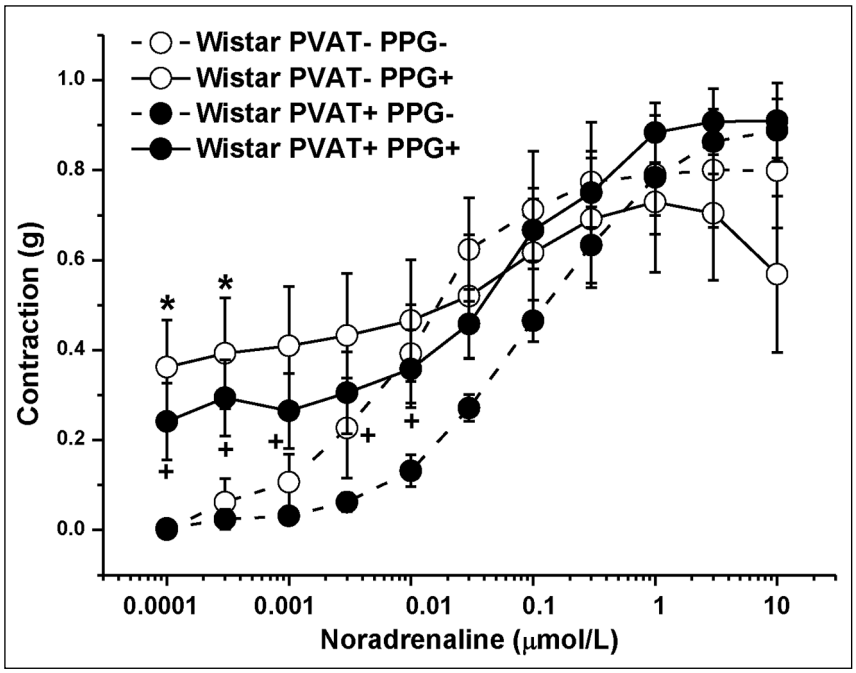

Fig. 4. Exogenous noradrenaline-induced contractile responses of mesenteric arteries without PVAT and intact PVAT before (PPG$^-$) and 20 minutes after (PPG$^+$) pretreatment with propargylglycine in Wistar rats. Values are ± SEM. n = 8; *P < 0.05 versus Wistar PVAT$^-$ PPG$^-$; $^+$P < 0.05 versus Wistar PVAT$^+$ PPG$^-$.

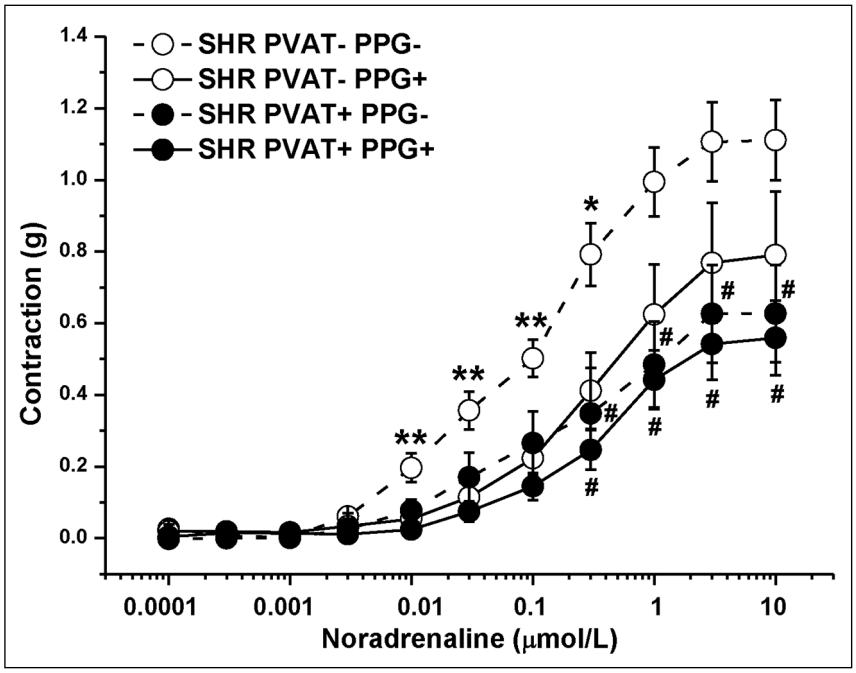

Fig. 5. Exogenous noradrenaline-induced contractile responses of mesenteric arteries without PVAT and intact PVAT before (PPG$^-$) and 20 minutes after (PPG$^+$) pretreatment with propargylglycine in SHR. Values are ± SEM. n = 8; *P < 0.05; **P < 0.01 versus SHR PVAT$^-$ PPG$^+$; $^\#$P < 0.05 versus SHR PVAT$^-$ PPG$^-$.

Effect of exogenous hydrogen sulfide

The application of Na_2S on NA-pre-contracted MA rings induced a dual effect in both experimental groups. Lower concentrations of Na_2S induced contraction (5 μmol/L in Wistar $PVAT^-$ rings, 5 – 40 μmol/L in Wistar $PVAT^+$ rings, 5 – 40 μmol/L in SHR $PVAT^-$ rings, 40 μmol/L in SHR $PVAT^+$ rings), whereas the higher concentrations evoked vasorelaxation of

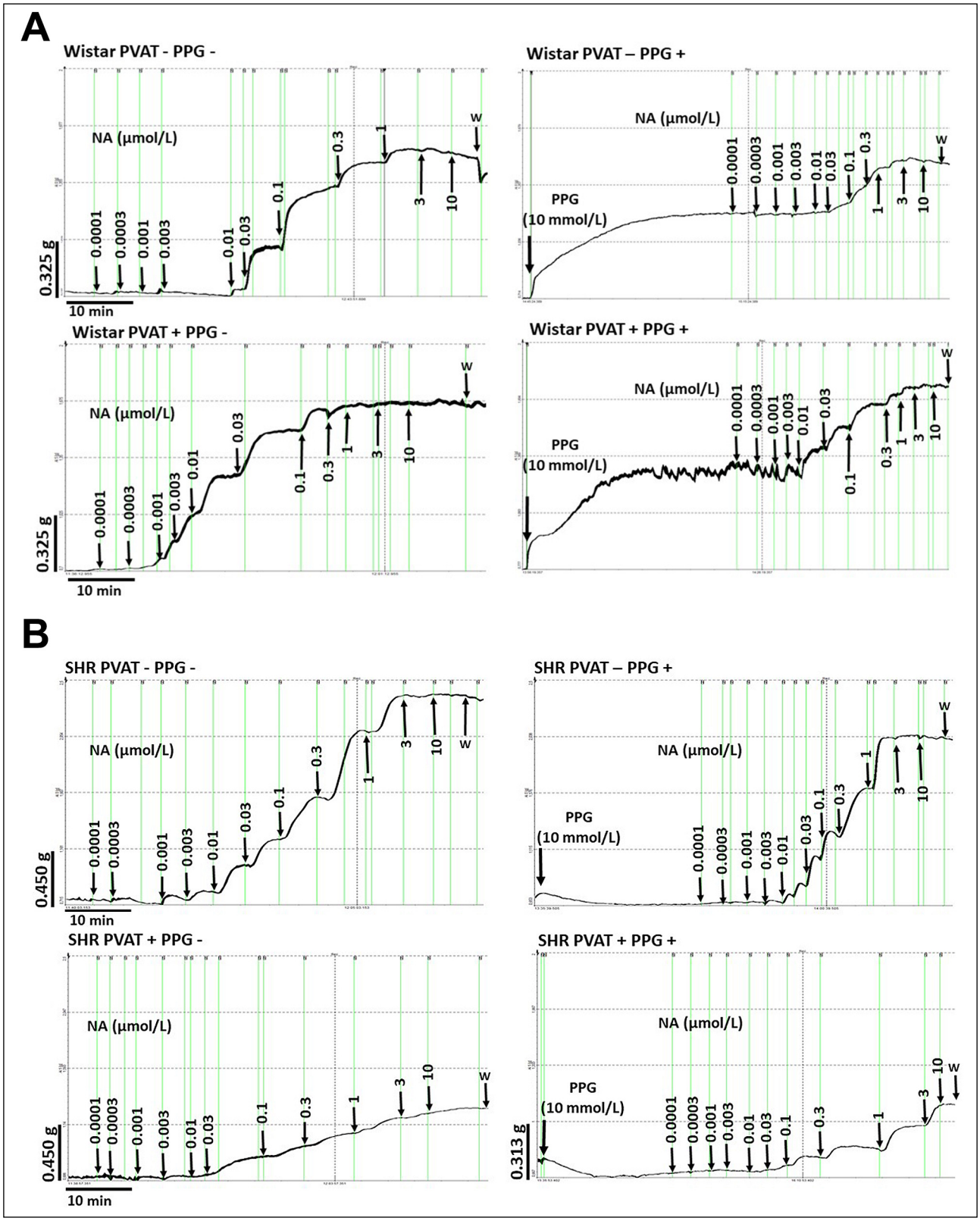

Fig. 6. Original record of the contractile responses induced by cumulative concentrations of exogenous noradrenaline (NA) in mesenteric arteries with intact ($PVAT^+$) or removed ($PVAT^-$) perivascular adipose tissue in Wistar rats (A) and SHR (B) before (left panels) and after (right panels) the acute pre-treatment with CSE inhibitor propargylglycine (PPG, 10 mmol/L). w = washout.

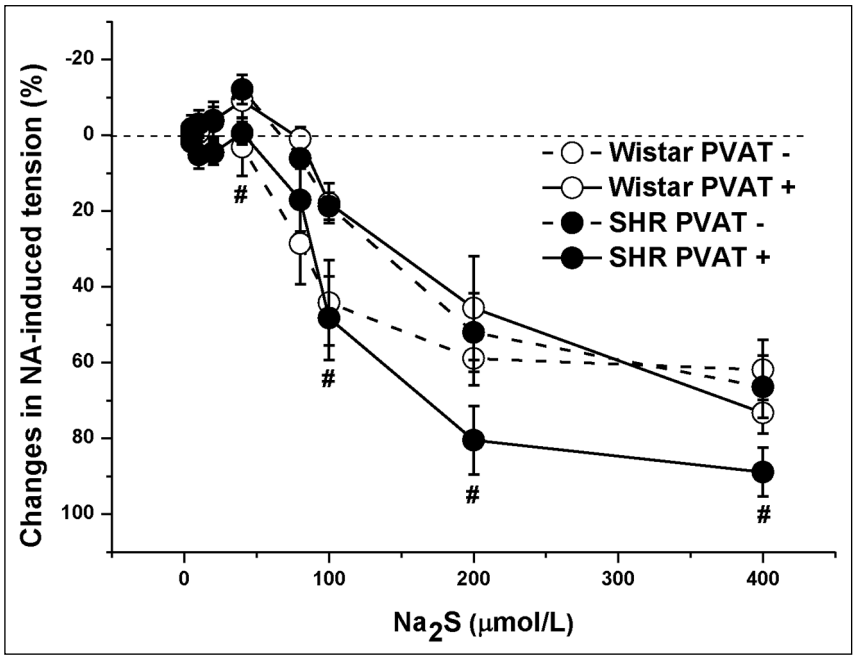

Fig. 7. Comparison of the vasoactive effects of exogenous H_2S donor (Na_2S) in mesenteric arteries without PVAT and intact PVAT in Wistar rats and SHR. Values are ± SEM. Wistar rats: n = 8; SHR: n = 8; $^{\#}P < 0.05$ versus SHR $PVAT^{-}$.

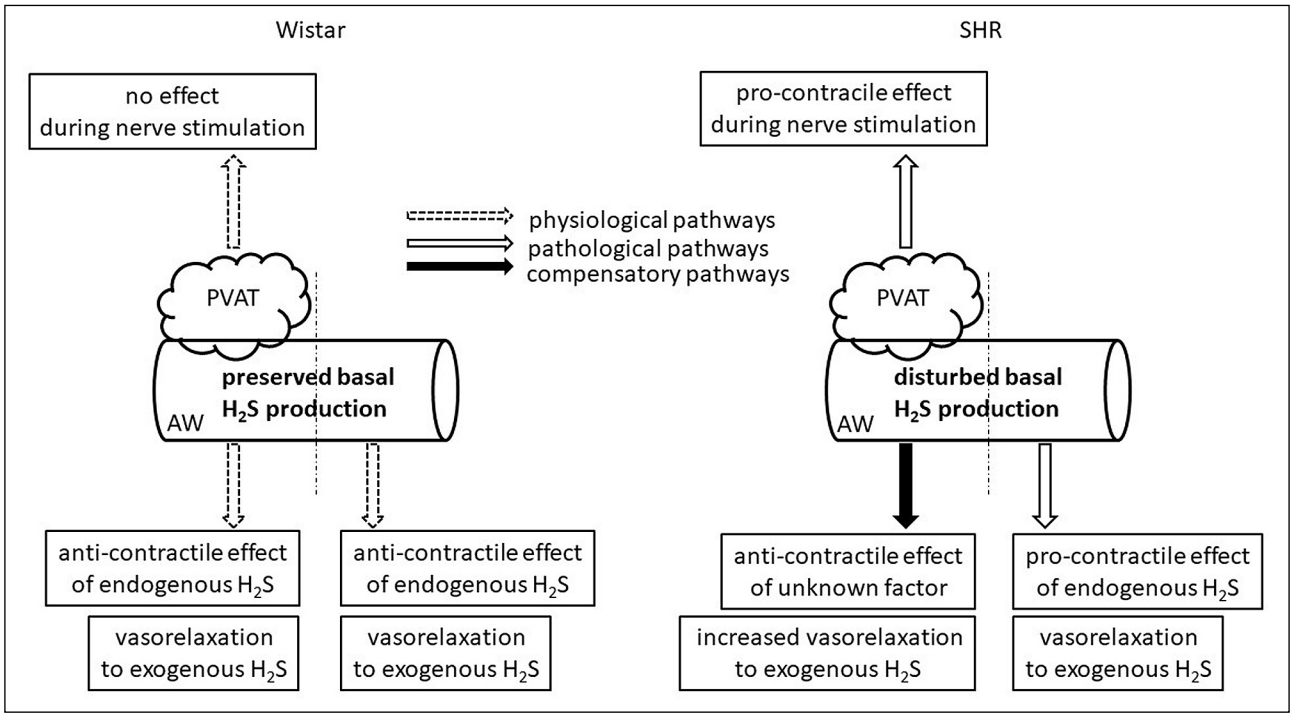

Fig. 8. Schematic illustration on interactions between PVAT and H_2S signaling in Wistar rats and SHR. PVAT and endogenously produced H_2S can manifest as pro-contractile or anti-contractile properties in rat mesenteric artery depending on the type of triggered signal pathway. In normotensive rat mesenteric arteries, the presence of PVAT did not affect the contractile response induced by perivascular nerve stimulation. The basal generation of H_2S by both the PVAT and arterial wall could be predominantly responsible for the anti-contractile effect of endogenous H_2S. The presence of PVAT did not affect the vasorelaxant response to exogenous H_2S. In mesenteric arteries of SHR, the pro-contractile effect of PVAT was closely associated with perivascular nerve stimulation. The disturbed basal H_2S production and the pro-contractile action of H_2S in the arterial wall could be balanced by compensatory vasoactive mechanisms triggered by PVAT: i) stronger anti-contractile action of an unknown factor (other than H_2S); ii) potentiation of the vasorelaxant effect of exogenous H_2S. PVAT, perivascular adipose tissue; AW, arterial wall.

arterial wall (*Fig. 7*). In normotensive Wistar rats the presence of PVAT did not affect significantly the vasoactive responses induced by Na_2S (*Fig. 7*). On the other hand, in SHR, the presence of PVAT significantly reduced the contractile part of Na_2S-induced vasomotor responses (at 40 μmol/L; $P < 0.05$) and increased the relaxant responses (100 – 400 μmol/L; $P < 0.05$; *Fig. 7*). We confirmed an interaction between PVAT × strain ($F_{(1,156)} = 23.55$, $P = 3.525 \times 10^{-6}$).

DISCUSSION

PVAT and vascular H_2S seem to be linked systems, which in cooperation with other regulatory units, may be involved in physiological and pathophysiological processes in arteries. Alterations of both systems may contribute to the increased vascular tone in hypertension. Nevertheless, our new findings show that under conditions of essential hypertension, the compensatory vascular mechanisms associated with stronger anti-contractile action of PVAT and PVAT-mediated potentiation of exogenous H_2S vasorelaxation could be triggered.

Anti-contractile properties of perivascular adipose tissue

In this study, we confirmed that in Wistar rats, visceral PVAT attenuated the contractions of MA to exogenous noradrenaline, although the attenuation was manifested as decreased sensitivity, whereas the maximal absolute contraction was not affected. These results are in accordance with the well-documented anti-contractile effects of PVAT under normotensive conditions (9). In SHR, where hypertension was associated with cardiac hypertrophy and increased contractility, the interplay of several factors could take part in the modulation of PVAT properties. Due to the lower amount of PVAT usually found in SHR (10), the release of anti-contractile substances could be limited so that contractions might be facilitated. On the other hand, Zemancikova and Torok demonstrated that moderate increase in body adiposity did not potentiate the anti-contractile effect of PVAT in SHR (17). In our study, we did not observe significant decrease of retroperitoneal fat; moreover, analogous to other studies, we detected the decreased level of triacylglycerols in the plasma in SHR compared to Wistar rats (10, 18). In the terms of such background, we confirmed a significant effect of the strain and a strain-PVAT interaction, SHR MA with intact PVAT surprisingly responded with lower intensity to exogenous noradrenaline compared to all other groups. Rather than with the content of body fat and/or lipid profile, an explanation for these results could be associated with the setting up of the basal tension. Galvez *et al.* (10) showed that in SHR, unlike in Wistar rats, the value of used pre-tension affected the anti-contractile effect of PVAT in MA. Authors confirmed that in SHR the anti-contractile properties of PVAT disappeared when higher value (2 mN/mm) of wall pre-tension was used than lower one (1.2 mN/mm). Zemancikova and Torok (9) used the same value of pre-tension in MA of both strains (1 g) and demonstrated a weaker anti-contractile effect of PVAT in SHR comparing to WKY rats. In our study, we used the same; however, lower value (0.7 g) of pre-tension in Wistar and SHR, which could strengthen the intensity of the anti-contractile effect in SHR. It seems that the anti-contractile effect of PVAT in SHR could be dependent on the wall pre-tension and that wall pre-tensions, which are closer to the wall tension *in vivo* could affect, probably diminish, the anti-contractile effect of PVAT. Nevertheless, this question requires further investigation.

Pro-contractile properties of perivascular adipose tissue

We recorded the difference in the action of PVAT when different sources of noradrenaline were tested. Unlike using exogenous noradrenaline, after transmural nerve stimulation we demonstrated the pro-contractile properties of PVAT in SHR. A detailed data evaluation of the frequency-response curves did not reveal an effect of strain. However, we confirmed a significant effect of PVAT. Torok *et al.* (20) showed in adult normotensive rats that most of the sympathetic nerve terminals are concentrated in the surface layers of the proper mesenteric arterial wall. In our study, we documented that the pro-contractile effect of PVAT arose in SHR during neurogenic contractions suggesting that active innervation could be present in PVAT too. Moreover, nerve stimulation in the arterial wall could be associated with the overproduction of pro-contractile factors. Lu *et al.* (21) and Gao *et al.* (22) showed in MA that PVAT promoted vasoconstriction to perivascular neuronal activation through the generation of angiotensin II and superoxide, and Knapp and Klann (23) confirmed that the presence of superoxide could induce long-lasting potentiation of synaptic transmission. Alvarez *et al.* (24) demonstrated increased superoxide formation from NADPH oxidase in vessels from SHR compared to normotensive rats. It seems that the increased density of sympathetic innervation in arterial wall and PVAT together with the increased oxidative stress, both generally demonstrated in SHR (25, 26), could lead to stronger pro-contractile action of PVAT triggered after stimulation of nerve endings in mesenteric arterial walls. PVAT provides a dynamic system that is able take up, metabolize and release noradrenaline also from non-neuronal sources (27). Weisberg *et al.* (28) confirmed the secretion of catecholamines by macrophages present in adipose tissue. Okruhlicova *et al.* (29) demonstrated an increased infiltration of macrophages in cardiovascular system of SHR and Thang *et al.* (30) showed in hypertensive animals that superoxide generated from infiltrating macrophages increased the release of noradrenaline from sympathetic nerves. Other groups supported the role of adipocytes in the synthesis of catecholamines (31). Ayala-Lopez *et al.* (32) used tyramine, an indirectly acting sympathomimetic drug, as a tool to test whether a functional pool of catecholamines existed in PVAT, and they confirmed that sympathetic nerves were not necessary for tyramine-induced PVAT-dependent contractions in normotensive Sprague Dawley rats. In our study, we confirmed that the presence or absence of PVAT did not affect tyramine-induced contractile responses in both strains. Only a significant effect of the strain was confirmed. Therefore, we suggest that the increased pro-contractile effect observed in SHR with PVAT was directly associated with the process of nerve impulse transport.

Perivascular adipose tissue - hydrogen sulfide interaction in Wistar rats

Many data were gathered about substances, which are produced by PVAT and are capable to affect the vascular reactivity. We focused on the role of H_2S that can be produced, in addition to PVAT (2), by arterial smooth muscle cells (3) and endothelial cells (4). Several authors confirmed that endogenous H_2S is engaged in basal tone and blood pressure regulation. Yan *et al.* (33) demonstrated that endogenous H_2S participated in basal tone regulation in normotensive rats since the administration of PPG (35 mg/kg) inhibited CSE gene expression and activity as well as endogenous H_2S production in the thoracic aorta, which was associated with blood pressure increase. In our study, the use of increasing concentrations of PPG or the pretreatment with the inhibitor led to the vasoconstriction of MA and elevated basal tone in Wistar rats (*Fig. 2*, *Table 2*). Moreover, it seems that the basal release of H_2S could be predominantly responsible for the anti-contractile effect in normotensive MA because PPG increased contractions already at starting concentrations of noradrenaline, and the maximal developed force remained unaffected (*Fig. 3*). On the other hand, a loss of endogenous CSE-generated H_2S was shown in the MA of SHR. This could be explained by the finding of Yan *et al.* (33), who found the decreased gene expression and activity of CSE in the arterial wall of SHR and the further down-regulation of H_2S had little effect.

Under normotensive conditions, the anti-contractile effect of H_2S produced by PVAT was confirmed. Schleifenbaum *et al.* (1) showed that incubation of the thoracic aorta with PPG significantly reversed the anti-contractile effect of PVAT suggesting that H_2S might act as an ADRF. Similarly, Fang *et al.*

(2) observed that pre-treatment with PPG significantly increased the vasoconstrictor responses induced by different vasoconstrictors in rat thoracic aorta with PVAT. Authors confirmed CSE protein expression in both the aorta (vascular smooth muscle cells) and PVAT (adipocytes), and that the PVAT-generated H_2S was a releasable vascular relaxation factor acting in a paracrine manner by opening the K_{ATP} channel in an NO–, endothelium-, and a Ca^{2+} channel-independent manner. Kohn *et al.* (34) showed that CSE inhibition with PPG in mouse aortas did not influence serotonin-induced contractions in PVAT intact arteries indicating that CSE-H_2S was not involved in anti-contractile effect of perivascular fat. Authors suggest that H_2S acted rather as modulator of ADRF than primary ADRF. In our study, we confirmed the anti-contractile effect of H_2S produced by both the PVAT and arterial wall; however, the statistical evaluation confirmed the importance of PVAT-PPG interaction indicating the crucial role of PVAT in the anti-contractile effect of H_2S in normotensive MA.

Perivascular adipose tissue - hydrogen sulfide interaction in spontaneously hypertensive rats

In MA isolated from SHR, we observed that H_2S produced by arterial tissue, but not PVAT, contributed to an adrenergic vasoconstrictor response by a pro-contractile effect, which predominantly issued from the ability to increase the sensitivity of arterial wall to noradrenaline. Beside other mechanisms, the vasoconstrictor action of H_2S may depend on the presence of endogenously synthesized NO (35). As mentioned above, we confirmed a loss of endogenous CSE-generated H_2S in SHR compared to Wistar rats. Low concentrations of H_2S were found to induce the down-regulation of L-arginine/NO pathway in both cultured and isolated vascular tissues *via* several mechanisms (7, 35). Similarly, our previous experiments using isolated thoracic aorta demonstrated in Wistar and SHR that the pre-treatment with L-NAME, an inhibitor of NO-synthase, diminished the contractile vasoactive effects of low concentrations of H_2S donor (14, 15). Recently, Szijarto *et al.* (36) demonstrated that one of the major roles of H_2S produced by CSE in the murine circulation is to reduce endothelial NO bioavailability by the direct H_2S and NO interaction. Authors also demonstrated that CSE-H_2S pathway was not involved in PVAT regulation of arterial tone. Although contradictory results regarding H_2S and NO interaction have been published (8, 35), we suggest that pro-contractile effect of low H_2S production in arterial wall of SHR could be associated with inhibitory effect on NO signaling.

Regarding the PVAT-related action little is known about the function of H_2S produced by PVAT during hypertension. In NO-synthase inhibited hypertensive rats and SHR, aortic CSE gene expression and H_2S production in the aorta and plasma were decreased (33, 37), nevertheless, authors did not evaluate the role of PVAT. In rats with pressure-overloaded hypertension induced by abdominal aortic banding, H_2S generation and CSE protein expression were significantly increased in PVAT, but not in aortic wall (2). The authors showed that transplanting PVAT into the periadventitia of stenotic aortas ameliorated the elevated arterial blood pressure and decreased the angiotensin II level in the aorta, probably due to the increased H_2S action. In our study, PVAT eliminated the pro-contractile effect of H_2S; nevertheless, the pre-treatment with PPG did not affect noradrenaline contraction farther. We suggest that PVAT of MA in SHR is able to trigger the production of an anti-contractile factor; however, it is probably not H_2S. Similarly, Kohn *et al.* (34) have demonstrated that CSE inhibition with PPG in mouse aortas did not influence the anti-contractile effects of PVAT, however, exogenous H_2S was able to induce a strong vasorelaxation. Because both exogenous H_2S and ADRF could utilize opening of KCNQ-type K_V channels authors suggested that KCNQ channel opening might serve as a promising target to induce relaxation in pathological stages associated with ADRF malfunction. Although we did not observe damaged anti-contractile properties of PVAT in hypertensive MA, our results are in good consent with this suggestion. We confirmed in SHR, unlike in Wistar rats, that the presence of PVAT significantly reduced the contractile part of Na_2S-induced vasomotor responses, and increased the vasorelaxant responses indicating a close relationship between vasorelaxant action of H_2S and PVAT in SHR. In our previous study, we have found that the H_2S donor regulated the increased tone of the thoracic aorta towards vasorelaxation significantly more in SHR than in normotensive rats (14). Moreover, this effect triggered in the early ontogeny period was enhanced in adulthood suggesting that the development of hypertension could potentiate H_2S-balancing properties (15, 38). We hypothesized that exogenous H_2S could take part in compensatory vasoactive responses under conditions of essential hypertension. The results of this study renewed our hypothesis: in essential hypertension, the decreased basal production of H_2S and the pro-contractile effect of H_2S in arterial wall could be counter-balanced by PVAT-dependent potentiation of H_2S vasorelaxantion. What is the mechanism and whether the interaction between anti-contractile properties of PVAT and up-regulation of H_2S action exists needs to be further investigated. Schematic illustration on interactions between PVAT and H_2S signaling in Wistar rats and SHR is described in the *Fig. 8*.

In summary, our findings suggest that PVAT-H_2S interaction may play an important role in the modulation of vascular function of both strains. Both PVAT and endogenously produced H_2S could manifest as pro-contractile or anti-contractile properties in rat MA depending on the type of triggered signal pathway. In SHR, unlike in Wistar rats, the pro-contractile effect of PVAT was closely associated with perivascular nerve stimulation, and besides the pro-contractile action of H_2S in the arterial wall, it could probably participate in the pathologic features of SHR. On the other hand, PVAT of hypertensive MA is probably endowed with compensatory vasoactive mechanisms, which include stronger anti-contractile action of a factor, probably other than H_2S, and potentiation of the vasorelaxant effect of exogenous H_2S.

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Author's address: Dr. Sona Cacanyiova, Department of Vascular Pathophysiology, Institute of Normal and Pathological Physiology, Centre of Experimental Medicine, Slovak Academy of Sciences, 1 Sienkiewiczova Street, 813 71 Bratislava, Slovakia
E-mail: sona.cacanyiova@savba.sk