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Adaptive vasoactive response to modulatory effects of endothelin-1 in spontaneously hypertensive rats

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Abstract:
In addition to a direct vasoconstrictor effect, endothelins modulate vascular responses induced by different mediators. We compared the effect of subthreshold concentrations of endothelin-1 (ET) on vasoreactivity of isolated pulmonary artery (PA) and on integrated blood pressure (BP) responses in both Wistar rats and spontaneously hypertensive rats (SHR). In one series of experiments (*in vivo*), after anesthesia the carotid artery was cannulated to measure mean BP. In another series of work (*in vitro*), the PA was isolated and changes in isometric tension were recorded. The subthreshold concentrations of ET *in vitro* (1 nM) and *in vivo* (0.1 nM) did not affect the basal tone of PA nor BP either in Wistar rats or in SHR. *In vitro* pretreatment of the PA with ET increased contraction in response to noradrenaline and decreased relaxation in response to acetylcholine in Wistar rats. In SHR, the effect of ET tended to reduce vasoactive tone in the PA: after the pretreatment with ET, contraction in response to noradrenaline was decreased and relaxation in response to acetylcholine was unchanged. *In vivo*, the bolus of ET did not change the integrated hypotensive response to acetylcholine in Wistar rats, while in SHR the response was increased. ET pretreatment did not affect the integrated pressor response to noradrenaline in Wistar rats nor in SHR. The effects of subthreshold concentrations of ET resulted in the increase of vasoactive tone in normotensive rats, while in SHR the potentiating effect of ET was impaired. An adaptive phenomenon to elevated arterial pressure could be one possible explanation for these results.

Key words:
endothelin-1, pulmonary artery, vasoactivity, spontaneously hypertensive rat

Abbreviations: BP – blood pressure, ET – endothelin-1, $-\log EC_{50}$ – negative logarithm of noradrenaline molar concentration producing half-maximal contraction, NO – nitric oxide, PA – pulmonary artery, SHR – spontaneously hypertensive rats

Introduction

Endothelins are potent vasoconstrictor substances that are produced in the vascular endothelium. They were found to restore normal blood pressure (BP) in hemorrhagic shock [20]. However, it could be supposed that they can contribute to abnormal systemic BP. The role of endothelins in spontaneous hypertension is still a matter of debate. In spite of the fact that plasma concentrations of endothelin-1 (ET) were significantly lower in spontaneously hypertensive rats (SHR) than those in age-matched normotensive rats [28], the vascular wall levels of endothelins were upregulated in the SHR [27]. In SHR, there was an increased release of ET in some particular vascular beds, such as mes-

enteric arteries [24]. At the same time, several studies documented the regional differences in the effects of endothelins on vascular tone and reactivity. Both vasoconstriction and vasodilation were seen in vascular beds, such as in the hindquarter and carotid artery and aorta, whereas other regions, including renal and mesenteric beds, responded to ET with vasoconstriction only [7, 33, 34]. ET has been shown to be a very important vasoconstrictor in pulmonary circulation; it induces vasoconstriction in isolated pulmonary artery (PA) preparations and increases pulmonary vascular resistance [6, 12, 16]. However, in spite of the fact that ET has been widely used as a vasoconstrictor in pulmonary circulation, this effect has not been observed at low concentrations [22, 31]. Moreover, it was found that in addition to the direct vasoactive action, the endothelins were able to modulate vascular responses induced by different mediators at subthreshold concentrations. Low doses of endothelins efficiently sensitize and augment the response to different vasoconstrictor substances in various vascular tissues of normotensive animals [13, 15, 31]. Zerrouk et al. [36] showed that subthreshold concentrations of exogenous ET also potentiated contractions induced by noradrenaline in the thoracic aorta of SHR.

No study has yet defined and compared the changes in vascular reactivity due to low concentrations of ET in large conduit arteries along with resistance vessels in normotensive rats and in SHR. The importance of endothelins in regulatory mechanisms of pulmonary circulation was emphasized and the PA is an integral part of pulmonary vascular bed. The aim of this study was to investigate and compare the effect of subthreshold concentrations of ET both on vasoactive responses of isolated PAs and on the integrated vasoactive response in normotensive (Wistar) rats and SHR. Part of this work was published as an abstract [4].

Materials and Methods

Experimental animals and treatments

Adult 18-week-old male normotensive Wistar rats (used as controls) and age-matched SHR were included in the present study in accordance with institutional guidelines and the procedures were approved by the State Veterinary and Food Administration of the Slovak Republic and by the Ethical Committee.

All rats were housed under a 12 h light-dark cycle at constant humidity and temperature, with free access to standard laboratory rat chow and drinking water. At the beginning of the experiment, systolic BP was measured noninvasively in pre-warmed rats by the tail-cuff plethysmographic method in both groups.

In vitro study

Animals (n = 9) were slightly anesthetized with diethyl ether and decapitated. The thoracic cavity was opened and the right and left main branches of extrapulmonary arteries were isolated, cleaned of connective tissue and cut into rings (3–4 mm in length). The rings were vertically fixed between two stainless steel wires – triangles in 20 ml incubation organ bath with Krebs solutions of the following millimolar composition: NaCl 118; KCl 5; $NaHCO_3$ 25; $MgSO_4$ 1.2; KH_2PO_4 1.2; $CaCl_2$ 2.5; glucose 11; ascorbic acid 1.1; $CaNa_2EDTA$ 0.032. The solution was oxygenated with 95% oxygen and 5% carbon dioxide and kept at 37°C. The upper wire triangles were connected to electromechanical transducers Sanborn FT 10 and potentiometric recorders (Labora) for recording changes in isometric tension. The resting tension was adjusted to 10 mN and applied to each ring. Subsequently, the preparations were allowed to equilibrate for 60–90 min until stress relaxation no longer occurred. At the end of each experiment, tissues were lightly blotted and their lengths and weights determined for calculation of their cross-sectional areas. Responses to contractile agonist were expressed as percent values of the maximum tissue response to the respective agonist. The extent of relaxation of arterial rings was expressed as a percentage of phenylephrine-induced contraction. Relaxant responses were followed on the rings precontracted with a submaximal dose of phenylephrine (10^{-5} M) to produce a stable plateau of contraction. The rings were then exposed to cumulative doses of acetylcholine ($10^{-8} – 3 \times 10^{-5}$ M). Contractile responses were induced by increasing concentrations of noradrenaline ($10^{-9} – 10^{-5}$ M) in a cumulative manner. The contraction and relaxation were also obtained in the presence of a subthreshold concentration of ET (1 nM). The rings had been pretreated with ET 30 min before contractile agents (phenylephrine or noradrenaline) were applied to incubating baths.

In vivo study

Animals (n = 6) in each group were anesthetized *ip* with ketamine and xylazine (0.25 ml and 0.1 ml/100 g b.w., respectively) and the right carotid artery was prepared, cannulated and connected to a pressure transducer for BP recording. During the next 15 min, the steady state was achieved and acetylcholine (0.1 and 1 μg) and noradrenaline (1 μg), each dissolved in 0.1 ml Krebs solution, were administered into the jugular vein (during 10 s). These experiments were carried out again after a 30 min pretreatment with ET (0.1 nM, dissolved in 0.1 ml Krebs solution, 10 s, *iv*).

Drugs

The following drugs were used: phenylephrine, acetylcholine, endothelin-1 (Sigma, USA), noradrenaline (Zentiva, Slovak Republic), ketamine, xylazine (Spofa, Czech Republic). All drugs were dissolved in distilled water; ET was dissolved in phosphate buffered saline (pH 7.2–7.4) containing 0.05% bovine serum albumin.

Data analysis

The data were expressed as the mean ± SEM. For the statistical evaluation of differences between groups, one-way analysis of variance (ANOVA) was used and followed by the Bonferroni *post-hoc* test. The differences in means were considered to be significant at $p < 0.01$. The values of concentration producing the half-maximum response (EC_{50}) were calculated from the individual dose-response curves and expressed as the negative logarithm of noradrenaline molar concentration.

Results

Basic parameters

Basic cardiovascular characteristics of the normotensive Wistar rats and SHR are shown in Table 1. The mean value of BP was significantly higher in SHR than in Wistar rats by approximately 53%. The increased BP in SHR was accompanied by significantly increased heart weight and heart weight-to-body weight ratio. The significant increase in relative heart weight in SHR suggested the occurrence of cardiac hypertrophy.

In vitro study

The concentration of ET (1 nM) negligibly affected the basal tone of the PA either in Wistar rats (0.30 ± 0.15 mN × mm^{-2}) or in SHR (0.34 ± 0.11 mN × mm^{-2}).

In PA precontracted by phenylephrine (10^{-5} M), the application of acetylcholine (10^{-8} – 3 × 10^{-5} M) in Wistar rats induced concentration-dependent relaxation, which reached 89.78 ± 4.50%. The addition of ET (1 nM) to the incubation medium inhibited the dose-dependent acetylcholine-induced relaxation of the PA in the range of concentrations 10^{-6} – 3 × 10^{-5} M. The maximum relaxation in the ET treated group represented only 51.86 ± 5.15% ($p < 0.01$, Fig. 1).

Tab. 1. Basic cardiovascular characteristics of Wistar rats and spontaneously hypertensive rats (SHR)

	Wistar	SHR
sBP (mmHg)	126.0 ± 2.0	192.4 ± 2.7*
BW (g)	358.77 ± 5.95	329.38 ± 7.37*
HW (g)	0.94 ± 0.01	1.14 ± 0.03*
HW/BW (mg/g)	2.62 ± 0.01	3.45 ± 0.04*

Values are expressed as the mean ± SEM; * $p < 0.01$ significant differences *vs.* Wistar; sBP – systolic blood pressure, BW – body weight, HW – heart weight, HW/BW – heart weight/body weight ratio

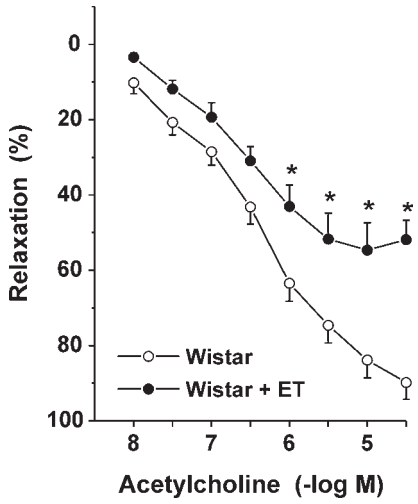

Fig. 1. Concentration-response curves to acetylcholine in pulmonary arteries from normotensive Wistar rats in the absence (Wistar; n = 9) and after 30 min pretreatment with 1 nM endothelin-1 (Wistar + ET; n = 9). Values are the mean ± SEM; * $p < 0.01$ significant differences *vs.* Wistar

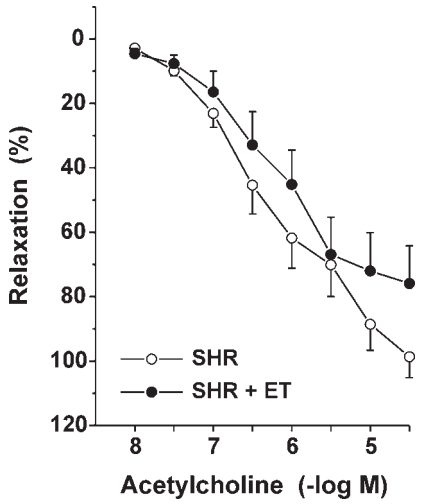

Fig. 2. Concentration-response curves to acetylcholine in pulmonary arteries from spontaneously hypertensive rats in the absence (SHR; n = 9) and after 30 min pretreatment with 1 nM ET (SHR + ET; n = 9). Values are the mean ± SEM

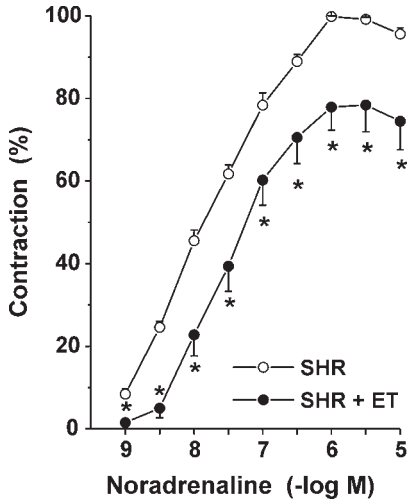

Fig. 4. Concentration-response curves to noradrenaline in pulmonary arteries from spontaneously hypertensive rats in the absence (SHR; n = 9) and after 30 min pretreatment with 1 nM ET (SHR + ET; n = 9). Values are the mean ± SEM; * $p < 0.01$ significant differences *vs.* SHR

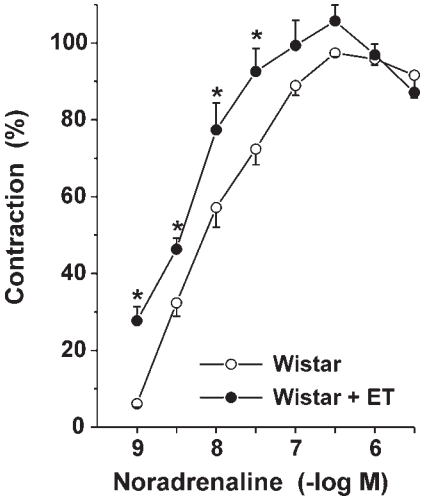

Fig. 3. Concentration-response curves to noradrenaline in pulmonary arteries from normotensive Wistar rats in the absence (Wistar; n = 9) and after 30 min pretreatment with 1 nM ET (Wistar + ET; n = 9). Values are the mean ± SEM; * $p < 0.01$ significant differences *vs.* Wistar

In SHR, acetylcholine ($10^{-8} – 3 \times 10^{-5}$ M) induced concentration-dependent relaxation in PA rings reaching 98.63 ± 6.52% was comparable to that observed in Wistar rats. Unlike in Wistar rats, in SHR the administration of ET (1 nM) to the organ bath did not change the relaxant response of PA to acetylcholine in the complete range of concentrations, and there was no significant difference in the maximal response between treated and untreated rats (Fig. 2).

The application of noradrenaline ($10^{-9} – 3 \times 10^{-6}$ M) to the incubation bath induced a dose-dependent contraction of the PA in normotensive Wistar rats with maximum at 3×10^{-7} M. The pretreatment with ET (1 nM) increased noradrenaline-induced contraction in Wistar rats. The dose-response curve was shifted to the left, thus indicating that the sensitivity of the PA to noradrenaline was increased compared to untreated arteries ($p < 0.01$; Fig. 3). In SHR, the increasing concentration of noradrenaline ($10^{-9} – 10^{-5}$ M) induced contraction of the PA. The maximum contraction was observed at a concentration of 10^{-6} M. The contraction of the PA in response to noradrenaline in the ET treated group was decreased in response to all concentrations, and the dose-response curve was shifted to the right when compared to the corresponding control, therefore indicating the decreased sensitivity of the PA to noradrenaline in ET pretreated arteries ($p < 0.01$; Fig. 4).

The values of the negative logarithm of noradrenaline molar concentration producing half-maximal contraction ($–\log EC_{50}$) are shown in Table 2. Half maximal contraction of the PA from Wistar rats occurred at significantly lower noradrenaline concentrations in the ET treated group than in the untreated group. On the other hand, a concentration of noradrenaline causing 50% of the maximal contraction of the PA from SHR was significantly higher in the ET treated group than in the untreated group. There was

Tab. 2. Parameters of the concentration-dependent responses to noradrenaline in the pulmonary artery

		Wistar	Wistar + ET	SHR	SHR + ET
Noradrenaline	$-\log EC_{50}$	8.33 ± 0.17	8.79 ± 0.18*	8.03 ± 0.07	7.37 ± 0.25^{+}
Noradrenaline	max (%)	97.22 ± 2.78	105.77 ± 4.12	99.85 ± 0.15	78.41 ± 8.93^{+}

Values are expressed as the mean ± SEM; * $p < 0.01$ significant differences *vs.* Wistar rats; $^{+}$ $p < 0.01$ significant differences *vs.* spontaneously hypertensive rats (SHR). $-\log EC_{50}$ – negative logarithm of the molar concentration of noradrenaline causing 50% of the maximal contraction; max – maximal contraction to noradrenaline (percentage); ET – endothelin-1

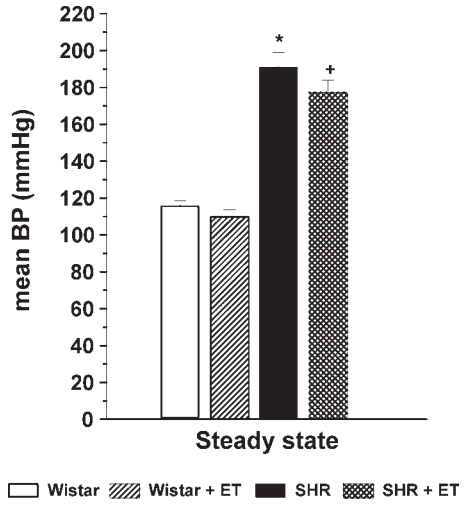

Fig. 5. Mean blood pressure (BP) in normotensive rats before (Wistar; n = 6) and after *iv* administration of endothelin-1 (ET) (0.1 nM in 0.1 ml Krebs solution in 10 s) (Wistar + ET; n = 6). Spontaneously hypertensive rats before (SHR; n = 6) and after *iv* administration of ET (0.1 nM in 0.1 ml Krebs solution in 10 s) (SHR + ET; n = 6) Values are the mean ± SEM; * $p < 0.01$ significant differences *vs.* Wistar; $^{+}$ $p < 0.01$ significant differences *vs.* Wistar + ET

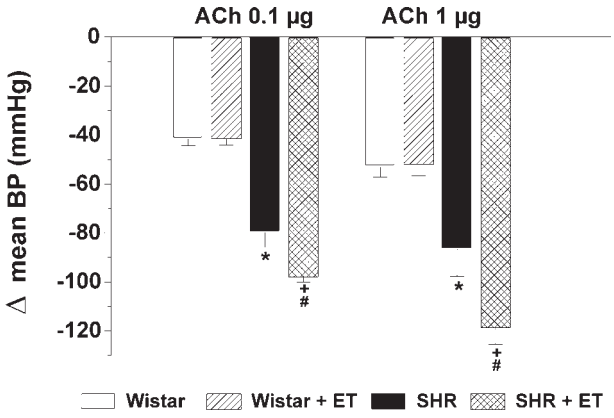

Fig. 6. Decrease of mean blood pressure (BP) after acetylcholine (ACh) administration (0.1 µg and 1 µg/0.1 ml Krebs solution in 10 s) in normotensive rats before (Wistar; n = 6) and after *iv* pretreatment with ET (0.1 nM in 0.1 ml Krebs solution in 10 s) (Wistar + ET; n = 6), and in spontaneously hypertensive rats before (SHR; n = 6) and after *iv* pretreatment with ET (0.1 nM in 0.1 ml Krebs solution in 10 s) (SHR + ET; n = 6). Values are the mean ± SEM; * $p < 0.01$ significant differences *vs.* Wistar; $^{+}$ $p < 0.01$ significant differences *vs.* SHR; $^{\#}$ $p < 0.01$ significant differences *vs.* Wistar + ET

no significant difference in relative maximal contraction in response to noradrenaline between untreated and ET treated normotensive Wistar rats. On the other hand, in SHR the relative maximal contraction to noradrenaline was significantly inhibited in ET treated rats compared to untreated rats.

In vivo study

Baseline values of mean arterial pressure were higher ($p < 0.01$) in SHR (191.14 ± 7.92 mmHg) compared to Wistar rats (115.99 ± 2.67 mmHg), and pretreatment with a subthreshold concentration of ET (0.1 nM) did not affect the mean arterial pressure either in Wistar rats (110.01 ± 3.7 mmHg) or in SHR (177.85 ± 5.94 mmHg; Fig. 5).

The hypotensive response to acetylcholine was increased ($p < 0.01$) in SHR (79.73 ± 6.14 mmHg at the dose of 0.1 µg; 86.59 ± 11.0 mmHg at the dose of 1 µg) compared to Wistar rats (41.64 ± 2.73 mmHg at the dose of 0.1 µg; 52.98 ± 4.27 mmHg at the dose of 1 µg). In Wistar rats, pretreatment with ET did not affect the hypotensive response to acetylcholine. On the other hand, in SHR pretreatment with ET increased the hypotensive response to acetylcholine and an augmented BP decrease was found ($p < 0.01$) at both doses of acetylcholine (98.18 ± 1.79 mmHg at the dose of 0.1 µg; 118.79 ± 6.67 mmHg at the dose of 1 µg; Fig. 6).

The pressor response to noradrenaline was increased in SHR (64.95 ± 4.06 mmHg) compared to Wistar rats (44.80 ± 2.98 mmHg). ET pretreatment

did not affect the pressor response to noradrenaline either in Wistar rats or in SHR (Fig. 7).

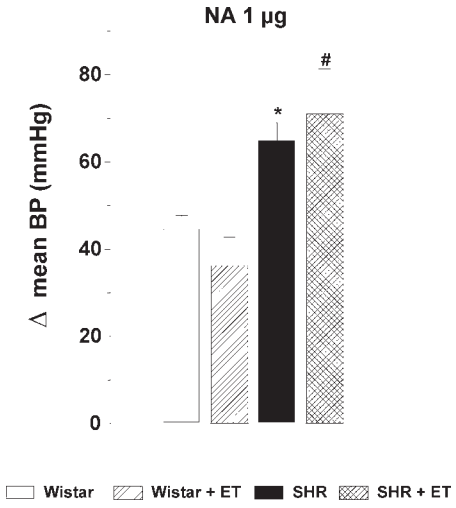

Fig. 7. Increase of mean blood pressure (BP) after noradrenaline (NA) administration (1 µg/0.1 ml Krebs solution in 10 s) in normotensive rats before (Wistar; n = 6) and after *iv* pretreatment with ET (0.1 nM in 0.1 ml Krebs solution in 10 s) (Wistar + ET; n = 6), and in spontaneously hypertensive rats before (SHR; n = 6) and after *iv* pretreatment with ET (0.1 nM in 0.1 ml Krebs solution in 10 s) (SHR + ET; n = 6). Values are the mean ± SEM; * $p < 0.01$ significant differences *vs.* Wistar; # $p < 0.01$ significant differences *vs.* Wistar + ET

Discussion

The modulatory effects of subthreshold concentrations of ET tended to an increase of PA vasoactive tone: the relaxant response induced by acetylcholine was decreased and the contractile response to noradrenaline was increased after pretreatment with ET. Similar findings were observed in guinea pig and rabbit PA, where low concentrations of ET potentiated the vasoconstrictor response to adrenergic stimulation in normotensive conditions [23, 31]. The fact that the sensitivity of PA to noradrenaline was increased after the pretreatment with ET is in good agreement with the finding that the potentiating effect of ET could be due to increased sensitivity of the contractile apparatus to Ca^{2+} [15]. Moreover, the effects of low concentrations of ET in normotensive conditions may be associated with the increased production of contractile cyclooxygenase-generated factors, mainly thromboxane A_2 [31], which also can participate in attenuation of vasodilation.

Studying the resistance arteries, we observed that under normotensive conditions the hypotensive response to acetylcholine and the pressor response to noradrenaline were unchanged after pretreatment with a low dose of ET. Thus, ET contributed to the modulation of vasoactivity in the conduit PA in a different manner than to the integrated vasoactive response, which predominantly represents reactivity of a resistant portion of the arterial tree. DeNucci et al. observed similar results [8], showing that in conduit arteries *in vitro* ET induced a reproducible and dose-dependent contraction, whereas BP measurements revealed that pressor activity of ET was limited by several factors (inactivation in lungs, etc.). Moreover, the importance of endothelins was underlined in mechanisms regulating pulmonary circulation, in which the PA takes part. There is evidence that ET binding sites are increased in the pulmonary circulation system and that ET induces and increases pulmonary vasoconstriction [19]. ET is produced and cleared in the pulmonary circulation system, and increased production of ET in the lung and the increased sensitivity of vascular receptors to ET correlates with increased pulmonary vascular resistance [9, 10].

Endothelium-dependent relaxation of the PA isolated from SHR was preserved and the magnitude of maximum relaxation to acetylcholine reached comparable levels to the normotensive controls. Moreover, the hypotensive response to acetylcholine was significantly increased in SHR compared to Wistar rats. We earlier observed an enhanced hypotensive response in SHR to another vasodilator agonist, bradykinin [11]. The increased endothelium-dependent relaxation in SHR was also observed in the aorta [26], in the femoral artery [2], and in the coronary artery [31]. Significant increase of endothelial nitric oxide (NO)-synthase proteins expression coupled with the NO-synthase activity was registered in the thoracic aorta of SHR [30]. Arnal et al. [1] observed similar levels of cyclic guanosine monophosphate in the aorta in Wistar-Kyoto rats and SHR. Even if the overproduction of vasoconstrictive agents contributed to the pathogenic mechanisms observed during hypertension, the vasodilator systems (such as NO) played an important role in counterbalancing this vasoconstrictor effect [3, 21]. Taken together, our observations confirm the idea that the vasodilatory system may be preserved and/or overactive in SHR and likely counteracts the pressor effect of the hypertrophied arterial wall.

Although the circulating endothelin levels were shown to be lower in SHR than those in Wistar rats [28], this may not exclude a role of endothelins in hypertension because the peptide is mainly released at the abluminal side of the vessel wall and a normal circulating level does not rule out locally increased levels [32]. Our results showed that in SHR the modulatory effect of subthreshold concentrations of ET lead to a reduction of PA vasoactive tone: after pretreatment with ET, the contractile responses to noradrenaline were decreased and the relaxant responses to acetylcholine was unchanged. Moreover, the hypotensive response induced by acetylcholine was increased and the pressor response remained unchanged after pretreatment with ET. It has been shown earlier that cardiac efficiency was not compromised in SHR between 10–20 weeks of age [35]. In addition, in our experiment, intravenously injected ET caused no changes in heart rate. Therefore, we supposed that the changes in systemic BP predominantly reflected the reactivity of resistance arteries. Our results revealed a similar trend in the modulatory effects of subthreshold concentrations of ET on vasoactivity in conduit and resistance arteries of SHR (decrease of vascular tone). From the point of view above, our results with subthreshold effects of ET also showed that during spontaneous hypertension, the mechanisms compensating for the negative impact of imbalance among vasoactive compounds could be activated to compensate for the consequences of long-term BP elevation. This is in agreement with results of Henrion et al. [14], which showed that in SHR, the chronic infusion of ET improved the flow-induced dilation of resistance mesenteric arteries due to increased production of cyclooxygenase vasodilator derivates. In addition, chronic ET administration increased the amplitude of flow-induced dilation through the increased participation of NO. Moreover, ET was shown to activate production of vasodilator cyclooxygenase product(s) in conductance [23] as well as in resistance arteries [17].

Further, Cargnelli et al. [5] detected a progressive decrease of contractile responses to ET with aging in aorta of SHR. The significant decrease of aortic responsiveness in SHR with aging might be due to chronic hypertension and indicate desensitization to ET. The latter might be related to chronic *in vivo* hyperproduction of endothelin, either genetically determined or related to the hypertension-induced endothelial damage. The local quantity of ET in the vascular wall can be upregulated in spontaneous hypertension [27] and the chronic action of increased endothelin levels may exert the feedback effect.

The different responses to ET observed between normotensive and SHR may be related to inherent differences between normal and hypertensive vessels, and it may be associated with the fact that the endothelin ET_A receptor and endothelin ET_B receptor mediated responses may differ in normotensive and hypertensive conditions. Itoh et al. [18] showed that a selective endothelin ET_A receptor antagonist inhibited the ET induced contraction of pulmonary arterial strips isolated from rats with pulmonary hypertension (hypoxic) more effectively than in normotensive rats, suggesting the downregulation of endothelin ET_A in rat PAs was not altered under hypertensive conditions.

Finally, as was shown in other animal models of hypertension, the predisposition of specific vascular areas to predominate vasodilation mediated by activation of endothelin ET_B endothelin receptors instead of vasoconstriction may present an additional compensatory mechanism against the increase in vasoactive tone. The lungs from chronically hypoxic rats had higher levels of endothelin ET_B receptors in the endothelium of the distal segments of the PAs [25]. In monocrotaline induced pulmonary hypertension, the luminal production of ET attenuated the increase in pulmonary vascular tone, suggesting the importance of local production of ET and the subsequent effect on endothelin ET_B receptors in the regulation of pulmonary vascular tone [10]. Taking into account all aforementioned facts, the upregulation of endothelin ET_B receptors in the endothelium, the desensitization or down-regulation of endothelin smooth muscle receptors, and/or increased production of vasodilation substances occurring after the long-term action of endothelins could be suggested as compensatory effects of subthreshold concentrations of ET in SHR.

Our results suggest that the subthreshold concentration of ET (i) participates in the regulation of BP and vasoactive tone without inducing irreversible long-term contraction in both normotensive rats and SHR, (ii) reveals the heterogeneity in modulatory effects between normotensive Wistar rats and SHR, (iii) leads to the increase of vasoactive tone in normotensive rats, and (iv) impairs the potentiating effect in SHR, which could be an adaptive response to elevated arterial pressure.

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