Effect of Chronic Treatment with Perindopril on Endothelium-dependent Relaxation of Aorta and Carotid Artery in SHRSP


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Abstract

Endothelium-dependent relaxation of aorta and carotid artery from stroke-prone spontaneously hypertensive rats (SHRSP) and the effect of chronic treatment of SHRSP with perindopril, an angiotensin converting enzyme inhibitor, on endothelium-dependent relaxation were studied. Endothelium-dependent relaxation was induced by acetylcholine (ACh) in preparations of SHRSP and normotensive Wistar Kyoto rats (WKY) precontracted with noradrenaline. The ACh-induced relaxation in both preparations was abolished by L-nitroarginine. The ACh-induced relaxation was impaired in preparations from SHRSP and contraction was observed at high concentrations of ACh. In the presence of indomethacin, impairment of endothelium-dependent relaxation in SHRSP was minimized and the contraction was inhibited. The relaxation with sodium nitroprusside did not differ between the preparations from WKY and SHRSP. Treatment of SHRSP with perindopril (2 mg/kg/day) for 6 weeks decreased systolic blood pressure and improved the ACh-induced relaxation of aorta and carotid artery. The treatment inhibited the contraction by higher concentrations of ACh in the presence of L-nitroarginine. These results indicate that the impairment of endothelium-dependent relaxation in aorta and carotid artery of SHRSP may be caused by the reduced availability of nitric oxide. The perindopril treatment may prevent these changes in SHRSP.

Key words: hypertensive rats, perindopril, aorta, carotid artery, endothelium, relaxation

Introduction

Spontaneously hypertensive rats (SHR) have been used widely in pathophysiological experiments. Interestingly, SHRSP have been used to study the effect of chronic treatment with perindopril on endothelium-dependent relaxation of aorta and carotid artery. This study investigated the impact of perindopril on the relaxation response of SHRSP aorta and carotid artery and compared it to that of normotensive Wistar Kyoto rats (WKY).

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studies on the mechanism of, and treatment for hypertension as a model of human hypertension. Endothelium-dependent relaxation of arteries from SHR has been shown to be impaired (Lüscher 1988; Lüscher and Vanhoutte, 1988; Sunano et al., 1989; Lüscher and Vanhoutte 1990; Vanhoutte and Boulanger 1995). The mechanisms of the impairment, however, have not been well understood. Reduced releases of endothelium-derived relaxing factor (EDRF, Malinski et al., 1993; Grunfeld et al., 1995; Hirata et al., 1996) and endothelium-derived hyperpolarizing factor (EDHF, Fujii et al., 1992; Fujii et al., 1993) and an increased release of endothelium-derived contracting factor (EDCF, Lüscher et al., 1992) have been proposed to contribute to the impairment. EDRF has been identified as nitric oxide (NO) and EDCF is thought to be thromboxane A2 and/or prostaglandin H2 (Kato et al., 1990; Dai et al., 1992; Mayhan 1992). The relative contribution of these abnormalities to the impaired relaxation in arteries from hypertensive rats may differ among the types of blood vessels.

It has also been reported that antihypertensive treatments of SHR prevented impairment of relaxation in arteries of SHR, or attenuated established impairment (Nigro et al., 1989; Clozel et al., 1990; Shimamura et al., 1991; Sunano et al., 1993; Dohi et al., 1994). It was also shown that chronic antihypertensive treatment of stroke-prone SHR (SHRSP), which had more severe hypertension than SHR, prevented the impairment of endothelium-dependent relaxation (Sunano et al., 1992; Shimamura et al., 1998). Among various treatments, chronic treatment with the angiotensin-converting enzyme (ACE) inhibitor has been reported to be the most effective (Clozel et al., 1990). Recently, treatment with perindopril, an ACE inhibitor, has been reported to have a strong effect to prevent the impairment of endothelium-dependent relaxation in the resistant arteries of SHR (Bennett et al., 1996). In the present study, the effects of chronic treatment of SHRSP with perindopril on endothelium-dependent relaxation of aorta and carotid artery were examined.

Materials and methods

Animals

Male stroke-prone spontaneously hypertensive rats (SHRSP) of Okamoto strain (Okamoto et al., 1974) and normotensive Wistar Kyoto rats (WKY) were used in the present experiments. They were successively bred in our animal facility with extensive care to maintain the respective desired blood pressures. Twenty seven WKY and 22 SHRSP were fed normal chow (SP, Funabashi, Japan) and water ad libitum under constant conditions of 22°C, 50% humidity and a 12 hour light and dark cycle. The systolic blood pressure of the rats was measured using a tail cuff method. Prior to the measurements, the rats were warmed at 40°C for 5 min.

In the experiment on the antihypertensive treatment of SHRSP, administration of perindopril was started at the age of 10 weeks. Perindopril was dissolved in water and the solution was administered (0.25 ml/100 g body weight) to 10 SHRSP via gavage at 1400 h everyday. The antihypertensive treatment consisted of perindopril at 2 mg/kg/day. The treatment was continued for 6 weeks. Six WKY and 8 control SHRSP were administered with same amount of water via gavage with the same schedule.
Preparations

Rats at the age of 16-17 weeks were bled from the vena cava under ether anesthesia and 200 ml of a modified Tyrode’s solution (warmed at 35°C) was perfused from the left ventricle. The thoracic aorta and carotid artery were excised and cleaned of fat and connective tissue in the modified Tyrode’s solution. From these aorta and carotid arteries, ring preparations of 1 mm width were made under a binocular microscope, taking care not to damage the endothelium. Endothelium-removed preparations of aorta and carotid arteries were made by rubbing the luminal surface of the preparations with a soft rubber band without damaging the underlying smooth muscle.

The composition of the modified Tyrode’s solution was (mM): NaCl, 137; KCl, 5.4; CaCl₂, 2.0; MgCl₂, 1.0; NaHCO₃, 11.9; NaH₂PO₄, 0.4 and glucose, 5.6; equilibrated with a gas mixture of 95% O₂ and 5% CO₂. The pH of the solution at 37°C was 7.3. High K-Tyrode’s solution (50 mM K) was made by replacing NaCl in the modified Tyrode’s solution with equimolar KCl.

Tension recording experiments

A tungsten wire (30 μm diameter) was inserted into the lumen of a preparation and the preparation was set on an apparatus which was immersed in an organ bath filled with the Tyrode’s solution. Another tungsten wire was connected to a force-displacement transducer (Minebea, UL-2GR, Karuizawa, Japan). Contraction or relaxation of the preparations was recorded as an isometric tension change. The initial passive tension was adjusted to 8 mN both in the preparations of aorta and carotid artery. After equilibration for 60 min in the modified Tyrode’s solution, the preparation was subjected to two successive 50 mM K-contractions of 30 min duration with an interval of 60 min. This procedure was required to obtain constant results in the subsequent experiments. The height of the second K-contraction was used to normalize the subsequent tension changes. At the end of the experiments, the preparation was relaxed by the application of verapamil (10⁻⁵ M) and papaverine (10⁻⁴ M) and tension was measured from this level.

Endothelium-dependent relaxation was induced by cumulative application of acetylcholine (ACH) to the preparation, which was precontracted in the presence of 5×10⁻⁷ M noradrenaline (NA). This concentration of NA has been shown to induce 80% of maximum tension (Osugi et al., 1990) and considered to be suitable to observe effect of EDRF and EDCF. The magnitude of relaxation was expressed in as the percentage height of the precontraction. Prior to the application of NA, 26 μM Ca-ethylenediamine tetraacetic acid (Ca-EDTA) was added to the organ bath so that the oxidation of NA was prevented.

The drugs used in the present experiments were: noradrenaline bitartrate (Sigma, St Louis, USA), acetylcholine HCl (Wako, Osaka, Japan), papaverine HCl (Wako), verapamil HCl (Ei-sai, Osaka, Japan), indomethacin (Wako), L-nitroarginine (Aldrich, Milwaukee, USA), sodium nitroprusside (Wako), Ca-EDTA (Wako) and perindopril (Servier, France).

The values obtained were expressed as mean±SE with the number (n) of animals (blood pressure and body weight) or preparations (tension recording experiments) in parentheses. Statistical analysis was performed with the Student’s t-test or ANOVA (StatView 4.5J, Abacus...
Post hoc analysis was performed with Scheffe's test. P values less than 0.05 were considered statistically significant.

Results

1. Body weight and blood pressure of the rats

The body weights of WKY and SHRSP at 16 weeks of age were 332.6±2.1 g (n=27) and 265.7±1.3 g (n=22), respectively. The body weights of WKY were greater than SHRSP. The systolic blood pressure of the rats at 16 weeks of age was 137.9±1.1 mmHg (n=27) and 250±0.9 mmHg (n=22), respectively, in WKY and SHRSP. The systolic blood pressure of SHRSP was higher than that of WKY.

2. Endothelium-dependent relaxation of the aorta and carotid artery

In the endothelium-intact aortic preparation contracted in the presence of 5×10⁻⁷ M NA, application of ACh induced concentration-dependent relaxation. The threshold concentration of ACh for the relaxation of aorta was 3×10⁻⁹ M, both in the preparations from WKY and SHRSP (Fig. 1). In the preparations from SHRSP, the relaxation induced by ACh of concentrations higher than 10⁻⁸ M was markedly smaller than that from WKY and a tendency for rebound contraction was observed at concentrations higher than 3×10⁻⁶ M.

In carotid arteries, the threshold concentration of ACh for the relaxation of preparations from WKY was 10⁻⁹ M, whereas that from SHRSP was 3×10⁻⁸ M. The maximal relaxation in the preparation from SHRSP achieved at 3×10⁻⁸ M was significantly smaller than that

![Graph showing relaxation vs. -log [ACh] for WKY and SHRSP.](image-url)

Fig. 1. Acetylcholine (ACh)-induced relaxation of the preparations of thoracic aorta from WKY and SHRSP. The preparations were precontracted in the presence of noradrenaline (5×10⁻⁷ M) and ACh was applied cumulatively. The relaxation amplitude was expressed as a percentage of the precontraction height. Asterisks indicate significant differences in the values of the preparation from WKY (*, P<0.05 and **, P<0.001). n=26 and 8 in aortas from WKY and SHRSP, respectively.
Fig. 2. The effect of endothelium-removal, L-nitroarginine and indomethacin on ACh-induced relaxation in carotid arteries from WKY and SHRSP. a) ACh-induced relaxation in noradrenaline-precontracted preparations from WKY and SHRSP. n=11 and 10 for WKY and SHRSP, respectively. b) Effect of ACh on endothelium-removed preparations precontracted by noradrenaline. n=6 and 5 for WKY and SHRSP, respectively. c) Effect of ACh on preparations precontracted by noradrenaline in the presence of L-nitroarginine. n=13 and 15 for WKY and SHRSP, respectively. d) Effect of ACh on preparations precontracted by noradrenaline in the presence of indomethacin. n=16 and 8 for WKY and SHRSP, respectively. e) Effect of ACh on carotid arteries in the presence of L-nitroarginine and indomethacin. n=14 and 12 for WKY and SHRSP, respectively. 10^{-4} M L-nitroarginine and 10^{-5} M indomethacin were applied 30 min before the application of noradrenaline, respectively. Others are the same as those in Fig. 1.

achieved at 3 × 10^{-6} M in the preparations from WKY (Fig. 2a). However, in the preparations from WKY, a tendency towards contraction appeared at ACh concentrations higher than 10^{-5} M, and the relaxation by ACh of higher concentrations was not significantly different from that
of SHRSP.

ACh did not induce any change in tension in the presence of $5 \times 10^{-7}$ M NA in endothelium-denuded carotid artery both from WKY and SHRSP (Fig. 2b). After application of L-nitroarginine in NA-precontracted preparations with an intact endothelium, the relaxation to ACh was completely abolished and a slight contraction was induced by high concentrations of ACh in preparation both from WKY and SHRSP (Fig. 2c).

The relaxation by ACh in carotid arteries from SHRSP was improved in the presence of indomethacin $10^{-8}$ M when the concentration of ACh was higher than $3 \times 10^{-9}$ M. The effect of indomethacin was less prominent in the preparations from WKY and improvement of relaxation was observed only at concentrations of ACh higher than $10^{-8}$ M. Thus, the difference in the relaxation between preparations from WKY and SHRSP was minimized in the presence of indomethacin (Fig. 2d). The ACh-induced contraction in the presence of L-nitroarginine was inhibited by $10^{-8}$ M indomethacin (Fig. 2c).

Sodium nitroprusside (SNP) inhibited the NA-induced contraction of endothelium-removed aorta concentration-dependently. SNP induced similar relaxation in preparations from WKY and SHRSP (Fig. 3). In endothelium-intact aorta in the presence of L-nitroarginine and indomethacin, papaverine inhibited NA-induced contraction. There was no difference in papaverine-induced relaxation between WKY and SHRSP (data not shown).

3. Effect of chronic treatment with perindopril on blood pressure and body weight of SHRSP

The systolic blood pressure levels of WKY, control SHRSP and perindopril-treated SHRSP aged 10 weeks (just before treatment started) were $135.0 \pm 0.7$ mmHg ($n = 6$), $207.9 \pm 1.5$ mmHg ($n = 8$) and $211.5 \pm 1.8$ mmHg ($n = 10$), respectively. There was no difference in systolic blood pressure between control SHRSP and perindopril-treated SHRSP. The blood pressure of them was significantly higher than that of WKY. The blood pressure of control SHRSP increased gradually with age, however, blood pressure of perindopril-treated SHRSP did not increase with age. After 12 weeks old of age, blood pressure of perindopril-treated SHRSP
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Fig. 4. Systolic blood pressure of WKY, control SHRSP and perindopril-treated SHRSP (perindopril SHRSP) between ages of 10 weeks and 16 weeks. Details of the treatment were described in the text. $n=6, 8$ and 10 for WKY, control SHRSP and perindopril-treated SHRSP, respectively. Blood pressure of control SHRSP and perindopril SHRSP was significantly higher than that of WKY ($P<0.001$). Values are mean±S.E.M. S.E.M.s were smaller than size of each symbol. Asterisks indicate the blood pressure of perindopril SHRSP was significantly lower than that of control SHRSP by ANOVA ($P<0.01$).

was significantly lower than that of control SHRSP of respective age. At the age of 16 weeks, the blood pressure of perindopril-treated SHRSP (201.2±5.8 mmHg, $n=10$) was significantly lower than that of control SHRSP (247.3±2.4 mmHg, $n=8$) (Fig. 4).

The age–dependent increase in the body weight of SHRSP was unchanged by the treatment with perindopril. The body weight of perindopril-treated SHRSP at the age of 16 weeks was 250.4±5.5 g ($n=10$), which was not significantly different from that of control SHRSP, 260.5±4.1 g ($n=8$).

Fig. 5. Effects of the treatment of SHRSP with perindopril on the endothelium-dependent relaxation of aorta (a) and carotid arteries (b). $n=26$ and 30 for aortas, and $n=14$ and 18 for carotid arteries from control and perindopril-treated SHRSP, respectively. ACh-induced relaxation was greater in preparations from perindopril-treated SHRSP (perindopril) than that from control SHRSP (control). * and ** indicate significant differences from the values of the preparations from control SHRSP. Others are the same as those in Fig. 1.
4. Endothelium-dependent relaxation and contraction of the preparations from perindopril-treated SHRSP

In $5 \times 10^{-7}$ M NA-precontracted aorta and carotid artery of WKY and control SHRSP, which were administered with water by gavage, ACh induced relaxation in similar amplitude as those observed in Fig. 1 and Fig. 2.

Treatment with perindopril improved the ACh-induced relaxation in aorta and carotid artery from SHRSP. The relaxation amplitude increased without changing the threshold concentration in both aorta (Fig. 5a) and carotid artery (Fig. 5b) from perindopril-treated SHRSP. The tendency of contraction at high concentrations of ACh was not remarkable in the preparations from perindopril-treated SHRSP. At concentrations higher than $3 \times 10^{-8}$ M, the relaxation by ACh in aorta from perindopril-treated SHRSP was greater than that from WKY. At concentrations higher than $3 \times 10^{-6}$ M the relaxation by ACh in the preparations of carotid artery from perindopril-treated SHRSP was greater than that from WKY. A contractile response to high concentrations of ACh in the presence of L-nitroarginine was observed in carotid artery from control SHRSP but not from perindopril-treated SHRSP (Fig. 6).

Discussion

Our previous study (Osugi et al., 1990) and the present study showed the relaxing response to ACh in aorta and carotid artery both from WKY and SHRSP was mediated by the release of a factor or factors from the endothelium since it was completely blocked by the removal of the endothelium. This endothelium-dependent relaxation has been shown to be impaired in the arteries from SHR (Lüscher 1988; Sunano et al., 1989; Lüscher 1990). The mechanisms of the impairment have been explained either by a decreased release of nitric oxide (Malinski et al.,...
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1993; Grunfeld et al., 1995; Hirata et al., 1996), an increased release of EDCF (Lüscher et al., 1992), which was thought to be a product or products of an arachidonic acid cascade via the cyclooxygenase pathway (Kato et al., 1990; Fu-Xiang et al., 1992; Mayhan 1992; Lüscher and Vanhoutte 1986; Diedrich et al., 1990), a decreased release of EDHF (Fuji et al., 1992; Fuji et al., 1993), or a combination of these changes.

In aorta and carotid arteries, the involvement of EDHF is less probable, because endothelium-dependent relaxation was abolished by the application of L-nitroarginine, which is known to block the synthesis of NO (Ishii et al., 1990; Moore et al., 1990). This indicates that the relaxation observed in the present experiment was mediated entirely by NO, although membrane hyperpolarization has been reported to be involved (Van de Voorde et al., 1992; Waldron and Garland 1994). Therefore, we can discuss the impairment of endothelium-dependent relaxation in these arteries from SHRSP, and the effects of the chronic treatment with perindopril, in relation to NO and EDCF.

It has been shown from direct measurement of NO in kidney perfusate that the release of NO by ACh is reduced in preparations from SHRSP (Hirata et al., 1996). It has also been reported that bradykinin-induced release of NO from the cultured endothelium originated from SHRSP is decreased (Malinski et al., 1993). However, pharmacological experiments revealed that the release of NO was unchanged (Ito and Carretero 1992; Sawada et al., 1994), and that the release of EDCF increased, in the blood vessels of SHR (Lüscher et al., 1992; Kato et al., 1990; Dai et al., 1992).

The possible increase in release of EDCF was also indicated in the present experiment with carotid arteries, in which high concentrations of ACh induced endothelium-dependent contraction. Since the contraction was abolished by the application of indomethacin, the EDCF is thought to be a product, or products, of an arachidonic acid cascade via the cyclooxygenase pathway, possibly prostaglandin H₂ (Mizuno et al., 1982; Flower 1974). The impairment of the endothelium-dependent relaxation in the carotid arteries of SHRSP disappeared in the presence of indomethacin, indicating the involvement of EDCF in the impairment of the relaxation in SHRSP carotid arteries. However, in the presence of L-nitroarginine, we observed that high concentrations of ACh induced contraction both in the preparations from WKY and SHRSP. Because the contraction height in the presence of L-nitroarginine was similar, release of EDCF may not be different between the preparations from WKY and SHRSP. This discrepancy may be explained by interaction between NO and EDCF as indicated in pharmacological (Auch-Schwell et al., 1992; Ito et al., 1991) and biochemical experiments (Kanner et al., 1992), although the details remain to be clarified.

In the mechanism of impaired endothelium-dependent relaxation, a decrease in the sensitivity or reactivity to NO, including cyclic GMP, may be excluded, since the relaxation to SNP was identical in the preparations from WKY and SHRSP. It has been reported that cyclic GMP production was slightly increased in the smooth muscle from SHR when compared with that from WKY (Papapetropoulos et al., 1994).

Perindopril is an ACE inhibitor and treatment with this drug lowered the blood pressure of hypertensive rats, including SHR (Bennett et al., 1996; DiNicolantonio and Doyle 1985; Richer et al., 1986; Levy et al., 1993; Thybo et al., 1994; Bennett and Thurston 1996). In the present
experiment with SHRSP, it was demonstrated that treatment with perindopril lowered blood pressure as reported by Devlin et al. (Devlin et al., 1995). The decrease in blood pressure was smaller than that in their report, although the same dose of the drug was given. This may be due to the severe hypertension of the SHRSP used in the present study (180-190 mmHg vs. 250 mmHg).

The endothelium-dependent relaxation both in the aorta and carotid arteries was improved by treatment with perindopril. The improvement shown in the present experiments may be explained by the reduced blood pressure. The lowering of blood pressure restores endothelium-dependent relaxation which has been impaired due to hypertension (Shimamura et al., 1991; Sunano et al., 1993; Dohi et al., 1994; Shimamura et al., 1998; Bennett and Thurston 1996; Van de Voorde et al., 1988), however, the present treatment lowered the blood pressure of SHRSP only to 201 mmHg, and it has been reported that endothelium-dependent relaxation in aorta of SHR, which showed similar blood pressure, is impaired to a much higher degree (Sunano et al., 1989; Kato et al., 1990; Lüscher and Vanhoutte 1986). Similar results have been reported in the carotid artery of SHR (Hongo et al., 1988; Lüscher et al., 1988). Thus, it is suggested that the improved endothelium-dependent relaxation is not only due to reduced blood pressure, but also to pressure-independent effect of the drug, although a different conclusion was reached in the effect of perindopril treatment on the resistant arteries of Goldblatt hypertensive rats (Bennett and Thurston 1996).

It has been reported that recovery from impairment of endothelium-dependent relaxation in the arteries of hypertensive rat is more prominent after treatment with ACE inhibitors than that with other antihypertensive drugs (Clozel et al., 1990; Sunano et al., 1992; Bennett et al., 1996). Angiotensin II may play some significant roles in the endothelial mechanism and, therefore, its abnormality may contribute to the impairment of endothelium-dependent relaxation in hypertensive rats. It has been suggested that treatment of SHR with ACE inhibitors improved endothelium-dependent relaxation not by decreasing the release of EDCF, but by increasing the release of EDRF (Clozel, 1991). Recently, it was reported that the NO release from the kidneys of SHRSP was increased after treatment with imidapril (Hirata et al., 1996) and that the cyclic GMP content of aorta of SHR was increased after treatment with ramipril or perindopril (Gohlke et al., 1993). Superoxide produced in such as arachidonic pathways has been shown to scavenge endothelial NO and increased production of superoxide has been shown to be responsible in decreased release of NO in SHRSP (Grunfeld et al., 1995). Ramipril increased NO production with concomitant decreased superoxide accumulation (Wiener et al., 1997). These reports may indicate that the amount of NO increased in preparations of perindopril-treated SHRSP in the present study. Furthermore, an increased relaxing response to endothelium-independent agonists has been reported in the aortas of antihypertensive-treated rats (Shultz and Raj 1989) and in the cerebral arteries of cilazapril-treated SHRSP (Yang et al., 1993). A similar mechanism may play a role in the greater relaxation of the preparations from perindopril-treated SHRSP.

Regarding changes in the release of EDCF, the present results suggest that treatment with perindopril reduced the release of EDCF, since the contractile response to ACh in the presence of L-NNA was abolished in the preparation from perindopril-treated SHRSP. It has been
proposed that the involvement of EDCF in the restoration of impaired endothelium-dependent relaxation by ACE inhibitors is less important compared with the increased action of NO (Clozel, 1991). Thus, in the present study with perindopril treatment, it is possible that improvement in NO-mediated relaxation is more important, although experiments with indomethacin may clarify this possibility.

In conclusion, it was shown that the endothelium-dependent relaxation of aorta and carotid arteries was impaired in SHRP when compared with that in WKY. The impairment is brought about by the decreased availability of NO and interaction with EDCF. Treatment with perindopril prevented the impairment by changes in mechanisms mediated by NO and EDCF. The interaction between the release of NO and EDCF, and the effects of perindopril treatment on the interaction, remain to be investigated.

References


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