Effects of Chronic Treatment with Carvedilol on Smooth Muscle Tone and Endothelium-Dependent Relaxation of Aorta in Stroke-Prone Spontaneously Hypertensive Rats

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Abstract

Effects of chronic treatment of stroke-prone spontaneously hypertensive rats (SHRSP) with carvedilol, an agent which has both α - and β -adrenoceptor blocking actions, on spontaneous muscle tone and on structural and functional abnormalities of endothelium were studied. The treatment of SHRSP with the drug of the dose of 30 to 200 mg/kg/day lowered the blood pressure significantly. Spontaneous muscle tone in endothelium-removed preparation disappeared by the treatment. Noradrenaline-induced contraction was depressed by the treatment in endothelium-intact preparation but not in endothelium-removed preparation. The treatment prevented the structural and functional abnormalities of endothelium. Similar results were obtained by the treatment with propranolol.

These results indicate that carvedilol prevented abnormal contraction of SHRSP aorta through protective effects on smooth muscle and endothelium. These effects may play roles in blood pressure lowering effect of carvedilol.

Key words: hypertensive rats, carvedilol, propranolol, aorta, endothelium, noradrenaline, smooth muscle tone

Abnormalities in vascular contraction have been reported in hypertension (Triggle and Laher, 1985). Aortic smooth muscle of spontaneously hypertensive rats, for example, shows spontaneous tension development (tone) which is rarely observed in normotensive rats (Noon *et al.*, 1978; Sada *et al.*, 1989a; Sunano and Shimamura, 1991; Sasaki *et al.*, 1993). The function of endothelium is also altered in the aorta of spontaneously hypertensive rats (Konishi and Su, 1983; Sugimoto *et al.*, 1988; Sunano *et al.*, 1989; Lüscher *et al.*, 1991).

Chronic antihypertensive treatment with angiotensin converting enzyme inhibitors prevented functional abnormalities in vascular smooth muscle (Sada *et al.*, 1989a, 1989b; Sunano and Shimamura, 1991; Sunano *et al.*, 1992) and endothelium (Clozel *et al.*, 1990; Clozel, 1991;

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Sunano *et al.*, 1992) of hypertensive rats. Impairment of endothelial function was prevented by chronic treatment with hydralazine (Sunano *et al.*, 1989; Nigro *et al.*, 1989; Shimamura *et al.*, 1991; Sunano *et al.*, 1993).

Antihypertensive treatment combination with other drugs of different mechanisms is considered to be effective because antihypertensive treatment via one mechanism leads to activation of other compensatory process. Recently multiple-action agents have been introduced for antihypertensive treatment (Rahnm, 1987; Prichar, 1992). Carvedilol is a β -adrenoceptor blocking agent with α 1-adrenoceptor blocking activity (Sponer *et al.*, 1987; Seki *et al.*, 1988; Hashimoto *et al.*, 1988, 1991; McTavish *et al.*, 1993). However, no report is available on the effect of chronic treatment of carvedilol on vascular function. In the present experiment, effects of chronic treatment with carvedilol on the tone of smooth muscle and endothelium-dependent relaxation in aorta of stroke-prone spontaneously hypertensive rats (SHRSP) were studied.

Materials and Methods

Materials

Stroke-prone spontaneously hypertensive rats (SHRSP) and normotensive Wistar Kyoto rats (WKY) were used in the present experiments. They were originally obtained from Dr. Okamoto (Okamoto *et al.*, 1974) and bred successively in our animal facility under the conditions of 25°C, 50% humidity and light and dark cycle of 12 hours. Great care was taken in breeding the animal to maintain expected blood pressure. Systolic blood pressure of the rats was measured by means of tail cuff method. Prior to the measurement, the rats were warmed at 40°C for 5 minutes. They were sacrificed at age of 16 weeks under anesthesia with ethyl ether.

Treatments of hypertensive rats with carvedilol were started at the age of weaning (5 weeks old) and continued until the experiments (16 weeks old). The drug was mixed with chow changing the dose. The doses were in the range of 30 mg/kg/day (5 weeks old) to 200 mg/kg/day (16 weeks old). Treatment with propranolol was also performed giving the drug of 100 mg/kg/day from 5 weeks to 16 weeks of age. Amount of drugs administered was estimated from drug-mixed chow weight consumed by rats. Further increment of the doses was not available because of losses of appetite and body weight.

Mechanical responses (contraction and relaxation) of blood vessel were observed using aortae of rats at 16 weeks old. Thoracic aorta was excised and ring preparations of 1 mm width were made. In about one third of the preparations, endothelium was removed by rubbing the inner surface with soft rubber band. These preparations were mounted in the organ bathes filled with a modified Tyrode's solution of following composition (mM): NaCl, 137; KCl, 5.4; CaCl₂, 2.0; MgCl₂, 1.0; NaHCO₃, 11.9; NaH₂PO₄, 0.4; glucose, 5.6. The solution was kept at 37°C and aerated with a gas mixture of 95% O₂ and 5% CO₂. High-K-Tyrode's solution was made by replacing NaCl in the solution with equimolar KCl, and Ca-free Tyrode's solution, by omitting CaCl₂ from the modified Tyrode's solution.

Methods

Tension changes of the preparations were measured isometrically with a force-displacement transducer (Shinkoh, Japan) under stretch tension of 8 mN. Changes in stretch tension around this range, taking difference in the wall thickness of the preparations from SHRSP and WKY into consideration, did not show significant influences on the contraction of the both preparations. Endothelium-dependent relaxation, however, became weaker at higher stretch tension in both preparations. The experiments were started after two successive high-K(50 mM)-contractures. This procedure was required to obtain the constant results in the experiments.

Scanning electron microscopic observation of endothelium was performed as follows. Vena cava was cut under ethyl ether anesthesia and animals were perfused from left ventricle with the modified Tyrode's solution at pressure of 70 mmHg for 10 minutes. The perfusing solution were then changed to 2.5% glutaraldehyde in phosphate buffer pH 7.4. Then, the aorta was dissected, opened lengthwise, and pinned on a Teflon sheet. The tissue was postfixed in 1% osmic acid (OsO_4) in 0.1 M phosphate buffer at room temperature. The tissue was then dehydrated through graded ethanol, critical point-dried in CO_2 , and sputter-coated with gold-palladium and observed by scanning electron microscope (Hitachi S-800, Japan).

Drugs

Drugs used in the present experiment were; carvedilol (Daiichi, Tokyo, Japan), propranolol·HCl (Sigma, St. Louis, USA), noradrenaline bitartrate (Sigma, St. Louis, USA), acetylcholine·HCl (Wako, Osaka, Japan), papaverine·HCl (Wako, Osaka, Japan), verapamil·HCl (Ei-sai, Osaka, Japan) and N^G -nitro-L-arginine (Aldrich, Wisconsin, USA).

Obtained data were expressed as mean $\pm$ SE and analyzed using Student's t-test, considering P values smaller than 0.05 to be statistically significant.

Results

Body weight of the rats.

Body weight of WKY and SHRSP at 16 weeks of age was 310.1 ± 6.4 g ($n=29$) and 259.4 ± 4.0 g ($n=28$), respectively. The body weight of WKY was greater than SHRSP ($p<0.001$). The body weight of the SHRSP treated with carvedilol and propranolol was 217.2 ± 8.4 g ($n=11$) and 242.4 ± 7.1 g ($n=10$), respectively. The body weight of treated SHRSP was slightly smaller than that of untreated SHRSP ($p<0.001$ in carvedilol-treated SHRSP and $p<0.05$ in propranolol-treated SHRSP).

Blood pressure of animals.

Blood pressure of SHRSP at 5 weeks old was already significantly higher than that of WKY ($P<0.001$) and rose steeply with aging and reached at high plateau level in 12 weeks of age. The blood pressure at the age of 16 weeks was 245.8 ± 2.9 mmHg ($n=28$). Rise of blood pressure of WKY with aging, on the other hand, was not prominent and the blood pressure at 16 weeks of age was 135.3 ± 1.2 mmHg ($n=29$). The difference in the blood pressure between

SHRSP and WKY at 16 weeks of age was significant ($p < 0.001$).

Carvedilol lowered the blood pressure in 3 weeks after starting the treatment. The blood pressure of the carvedilol-treated and propranolol-treated SHRSP was 199.8 ± 1.9 mmHg ($n = 11$) and 208.1 ± 5.4 mmHg ($n = 10$), respectively, being significantly lower than that of untreated SHRSP ($p < 0.05$) at 16 weeks old of age (Fig. 1a, b).

Spontaneous tension development

Endothelium-removed preparation from 16 weeks old SHRSP exhibited spontaneous tension development (tone) in the absence of stimulants. The tone was dependent of extracellular Ca^{++} . It disappeared by the removal of extracellular Ca^{++} or by the application of verapamil (10^{-6}M). On the contrary, the addition of Ca^{++} to Ca^{++} -deprived medium induced tension development Ca^{++} -concentration dependently. The tone was depressed by endothelium and only weak tone was observed in endothelium-intact preparation (Fig. 2a). In aortic preparation of WKY, little tone was observed in endothelium-intact or -removed preparations (Fig. 2b).

Effects of carvedilol-treatment on the tone of aortic smooth muscle was studied using endothelium-removed preparation of SHRSP. The treatment prevented the development of spontaneous tone. Only small tone was observed in endothelium-removed preparation from carvedilol-treated SHRSP (Fig. 3a). Similar effect was observed by the treatment of SHRSP with propranolol (Fig. 3b).

Noradrenaline-induced contraction

Noradrenaline induced concentration-dependent contraction both in the endothelium-intact and -removed preparations from SHRSP and WKY. The maximal contraction was achieved at 10^{-5}M in the preparations both from SHRSP and WKY. No significant difference

Fig. 1. Effects of treatment with carvedilol (a), and propranolol (b) on the systolic blood pressure of SHRSP. The treatment was started at 5 weeks of age and their doses were determined to keep blood pressure under 200 mmHg at 16 weeks of age as described in the methods. Mean $\pm$ SE of 10 rats, respectively. Asterisks indicate significant differences from untreated control rats ($P < 0.05$).

Fig. 2. Dependence of intrinsic active tone on extracellular Ca and its depression by endothelium in aorta from SHRSP (a) and WKY (b). endothel (+): endothelium-intact preparation (n=6 to 9). endothel (-): endothelium-removed preparation (n=20 to 22). Asterisks indicate significant differences from endothelium-intact preparation (*, $P < 0.05$ **, $P < 0.01$).

Fig. 3. Effects of treatment of SHRSP with carvedilol (a) and propranolol (b) on intrinsic active tone of endothelium-removed preparation at different extracellular Ca concentration. Note that both treatments depressed the active tone to similar level to that of WKY aorta shown in Fig. 2. n=20 in untreated, 14 in carvedilol-treated and 11 in propranolol-treated rats, respectively. Asterisks indicate significant differences from control ($P < 0.01$).

in the developed tension between these preparations was observed (Fig. 4).

Endothelium depressed noradrenaline-induced contraction markedly. Endothelium depressed the contraction in the preparation from WKY more prominently than SHRSP (Fig. 4).

In endothelium-intact preparation of SHRSP, carvedilol-treatment depressed the nora-

Fig. 4. Dose-response curve for noradrenaline-induced contraction in endothelium-intact and -removed preparation. (a) aorta of SHRSP. (b) aorta of WKY. endothel (+): endothelium-intact preparation. endothel (-): endothelium-removed preparation. $n=16, 11, 9$ and 13 in endothelium-intact, -removed preparation from SHRSP aorta and endothelium-intact, -removed preparation from WKY, respectively. Asterisks indicate significant differences from endothelium-intact preparation ($P < 0.01$).

Fig. 5. Effects of treatment of SHRSP with carvedilol (a) and propranolol (b) on noradrenaline-induced contraction of endothelium-intact preparation. $n=8$ and 11 in preparations from carvedilol- and propranolol-treated SHRSP, 16 in untreated control SHRSP. Asterisks indicate significant differences from preparations of untreated SHRSP (*, $P < 0.05$; **, $P < 0.01$).

drenaline-induced contraction and shifted the concentration-response curve to the right (Fig. 5a). Similar depression was observed by the treatment with propranolol. The depression of the contraction was greater than that in carvedilol-treatment (Fig. 5b).

Spontaneous tone of the preparation was depressed by the carvedilol or propranolol treatment. There was no remarkable effect of the treatments on noradrenaline-induced contraction in endothelium-removed preparation.

High- K^+ -induced contraction

When K^+ -concentration of the Tyrode's solution was increased, aortic preparation contracted in a concentration-dependent manner. In endothelium-intact preparation, high- K^+ -

Fig. 6. Effects of treatment of SHRSP with carvedilol and propranolol on high- K^+ -induced contraction. (a) endothelium-intact preparation. (b) endothelium-removed preparation. Number of preparations for each point is between 12 and 25. Asterisks indicate significant differences from untreated SHRSP ($P < 0.05$).

Fig. 7. Acetylcholine-induced endothelium-dependent relaxation of aortic preparation from SHRSP and WKY. Aortic preparations were precontracted by 5×10^{-7} M noradrenaline and, after the contraction achieved its maximum, acetylcholine was applied cumulatively. The relaxation by acetylcholine was expressed as percentages of the contraction induced by noradrenaline. $n=21$ and 18, in the preparation from WKY and SHRSP, respectively. Asterisks indicate significant differences from WKY (**, $P < 0.01$).

Fig. 8. Effects of treatment of SHRSP with carvedilol (a) and propranolol (b) on acetylcholine-induced relaxation in endothelium-intact preparation. The experiment was performed and expressed same as described in Fig. 7. $n=18, 12$ and 8 in control, carvedilol treated and propranolol treated SHRSP respectively. Asterisks indicate significant differences from control (*, $P<0.05$ **, $P<0.01$).

Fig. 9. Scanning electron-micrographs of endothelial surface of aorta of untreated SHRSP (A), carvedilol-treated SHRSP (B) and propranolol-treated SHRSP (C). The treatments of the rats were same as described in the methods. Note the rough surface with crater formation in the endothelium of SHRSP and their protection by the treatments.

induced contraction of SHRSP aorta did not differ markedly from that of WKY aorta, although the sensitivity to lower concentration of K^+ was increased slightly in SHRSP (data not shown). Treatment of SHRSP with carvedilol decreased the high- K^+ -induced contraction slightly (Fig. 6a). The removal of endothelium increased the amplitude of the contraction. Carvedilol treatment did not change the contraction significantly in the endothelium-removed preparation (Fig. 6b). Similar effects were observed by the treatment with propranolol.

Endothelium-dependent relaxation

Endothelium-dependent relaxation was observed by applying acetylcholine in the preparation precontracted with noradrenaline of submaximal concentration (5×10^{-7} M). Acetylcholine relaxed the contraction dose-dependently. The relaxations were abolished by the removal of endothelium or in the presence of N^G -nitro-L-arginine in the preparations both from SHRSP and WKY. The acetylcholine-induced relaxation was significantly smaller in the aorta from SHRSP when compared with that in the aorta from WKY. The threshold concentrations of acetylcholine for the relaxation were not different (Fig. 7).

Treatment with carvedilol caused an enhancement of the acetylcholine-induced relaxation in SHRSP. The threshold concentration for the relaxation was not altered by the treatment. Similar effects were observed by the treatment with propranolol (Fig. 8).

Structural changes of endothelium

Endothelial surface of the preparation from 16 weeks old SHRSP was rough, and protrusion toward the lumen and crater formation were often observed. Treatment with carvedilol or propranolol prevented these abnormal changes and kept the surface flat and smooth (Fig. 9).

Discussion

The smooth muscle of aorta from SHRSP exhibited spontaneous tension development (tone) in the absence of stimulant as has been reported in SHR arterial smooth muscle (Noon *et al.*, 1978; Lindner and Heinle, 1987; Fitzpatrick and Szentivanyi, 1980). The tone is thought to be brought about by Ca^{++} influx through voltage-dependent Ca^{++} channel of the smooth muscle membrane, since it was abolished by the removal of extracellular Ca^{++} or by the application of a Ca -antagonist, verapamil. We have observed that intracellular Ca^{++} level is elevated in this preparation (Sasaki *et al.*, 1993) as has also been reported in cultured smooth muscle cells of SHR aorta (Sugiyama *et al.*, 1986, 1990). Above hypothesis is supported also by the evidence that Ca^{++} dependency of skinned smooth muscle is not altered in arteries of spontaneously hypertensive rats including SHRSP (Nghiem and Rapp, 1983; Mrwa *et al.*, 1986; Dohi *et al.*, 1990). The tone is easily observed in endothelium-removed preparation. Endothelium-derived relaxing factor (EDRF) or endothelium-derived hyperpolarizing factor (EDHF) would depress the tone (Martin, 1988; Suzuki and Chen, 1990; Lüscher and Vanhoutte, 1990).

It has been reported that the development of the tone in vascular smooth muscle of hypertensive rats can be prevented by treating the rat with angiotensin converting enzyme (ACE) inhibitor or by hydralazine (Sada *et al.*, 1989a, 1989b; Sunano and Shimamura, 1991;

Sunano *et al.*, 1992). In the present study, we observed that the development of the tone could also be prevented by the treatment with carvedilol or propranolol.

Noradrenaline-induced contractions of the endothelium-removed preparation indicated that the adrenoceptor of the smooth muscle were not greatly different between the preparation from SHRSP and WKY. In endothelium-intact preparation, on the other hand, marked difference was observed in the contraction. Endothelium depressed the noradrenaline-induced contraction in preparations both from SHRSP and WKY, however, the depression was more remarkable in the preparation from WKY (Osugi *et al.*, 1990 ; Sunano *et al.*, 1992). This result may indicate that release of EDRF by adrenergic agonists (Martin, 1986, 1988 ; Miller *et al.*, 1988 ; Kaneko and Sunano, 1993) was decreased in the aorta of SHRSP.

The EDRF released spontaneously or released by noradrenaline is thought to be nitric oxide (NO) (Kaneko and Sunano, 1993). Chronic treatment with carvedilol depressed noradrenaline-induced contraction and shifted the dose-response curve to the right in endothelium-intact preparation but not in endothelium-removed preparation. It can therefore be assumed that the depression of noradrenaline-induced contraction by the treatment is due to the change in the function of endothelium and not in that of smooth muscle. Thus, carvedilol-treatment could depress the noradrenaline-induced contraction in endothelium-dependent manner.

Treatment of SHRSP with carvedilol or propranolol depressed the dose-response curve for high-K⁺-induced contraction of endothelium-intact preparation slightly. No influence of the treatment was observed in endothelium-removed preparation. These may indicate that the treatments did not affect activation of voltage-dependent Ca channels.

The endothelium-dependent relaxation has been shown to be impaired in aorta of spontaneously hypertensive rats (Konishi and Su, 1983 ; Winqvist, 1988 ; Sunano *et al.*, 1989 ; Lüscher *et al.*, 1991). We also ascertained that the acetylcholine-induced endothelium-dependent relaxation was impaired in SHRSP aorta. The prevention of the impairment of endothelium-dependent relaxation by antihypertensive treatment has been reported with hydralazine (Sunano *et al.*, 1989 ; Shimamura *et al.*, 1991 ; Sunano *et al.*, 1993) and ACE inhibitors (Clozel *et al.*, 1990 ; Clozel, 1991 ; Shimamura *et al.*, 1991 ; Sunano *et al.*, 1992), where blood pressure of hypertensive rats was kept as low as that of normotensive rats. Present results that carvedilol-treatment prevented the impairment of the relaxation indicate that the impairment could also be prevented by the antihypertensive treatment with adrenergic blocking agents. It is noteworthy that the similar degree of endothelium-dependent relaxation to that of WKY aorta could be obtained in the aorta from carvedilol- or propranolol-treated SHRSP, although blood pressure was kept similar to that of SHR in which endothelium-dependent relaxation has been reported to be impaired (Konishi and Su, 1983 ; Sugimoto *et al.*, 1988 ; Sunano *et al.*, 1989 ; Lüscher *et al.*, 1991). Thus, the possibility of the involvement of other factors such as adrenergic receptors in the improvement of the endothelium-dependent relaxation was suggested, although further studies were required to reach a conclusion.

Endothelium of the arteries of spontaneously hypertensive rats has been known to be structurally altered (Hazama *et al.*, 1979 ; Limas *et al.*, 1980 ; Lüscher and Vanhoutte, 1990 ; Sunano *et al.*, 1993). The impairment of endothelium-dependent relaxation in SHR and SHRSP aorta was accompanied with the structural abnormalities of the endothelium as has

been reported in arteries of SHR (Lüscher, 1988; Sunano *et al.*, 1993). The structural abnormalities of the endothelium can be prevented by antihypertensive treatment (Limas *et al.*, 1983; Sunano *et al.*, 1993). In the present experiment, it was demonstrated that the abnormalities could be prevented by the treatment with carvedilol or propranolol. As the blood pressure of SHRSP treated by carvedilol or propranolol was kept much higher than that of WKY, this also suggests the possibilities of involvement of adrenergic receptor in preventing structural abnormalities of endothelium.

In conclusion, chronic antihypertensive treatment with carvedilol decreased spontaneous muscle tone and noradrenaline-induced contraction while increased acetylcholine-induced relaxation in SHRSP aorta. As endothelium as well as smooth muscle was responsible for these changes, these results indicated carvedilol-treatment affected both endothelium and smooth muscle. All of these changes may play roles in lowering blood pressure of SHRSP under carvedilol-treatment.

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