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Dietary cholesterol enhances impaired endothelium-dependent relaxations in aortas of salt-induced hypertensive Dahl rats

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Abstract

We investigated the effect of hypercholesterolemia on the vascular reactivity of thoracic aortas isolated from hypertensive Dahl salt-sensitive (DS) rats. DS rats were fed on a low-sodium diet (control group), a low-sodium plus high-cholesterol diet (CHOL group), a high-sodium diet (NaCl group) or a high-sodium plus high-cholesterol diet (NaCl + CHOL group) for 8 weeks. Hypercholesterolemia developed in the CHOL and NaCl + CHOL groups, while hypertension developed in the NaCl and NaCl + CHOL groups, with these changes being greatest in the NaCl + CHOL group. Aortic cholesteryl ester accumulation was observed only in the NaCl + CHOL group. Endothelium-dependent relaxations in response to acetylcholine were significantly attenuated in the aortic rings from the NaCl and NaCl + CHOL groups, compared to the control group. The degree of attenuation in the NaCl + CHOL group was significantly greater than that in the NaCl group. Endothelium-dependent relaxations induced by the calcium ionophore A23187 were attenuated only in the NaCl + CHOL group. Endothelium-independent relaxations in response to sodium nitroprusside were slightly but significantly attenuated in the NaCl + CHOL group. The relaxations in the CHOL group were comparable to those in the control group. These findings indicate that cholesterol feeding strikingly enhances the impaired endothelium-dependent relaxations and the slightly impaired endothelium-independent relaxations in the aorta of DS rats with salt-induced hypertension, parallel to the development of hypertension, hypercholesterolemia and cholesterol deposition.

Keywords: Dahl salt-sensitive rat; Endothelium-dependent relaxation; High-cholesterol diet; Hypercholesterolemia; Hypertension

1. Introduction

Both hyperlipidemia and hypertension are well-known risk factors in the development of atherosclerosis. In hypercholesterolemia, many studies have shown that endothelium-dependent relaxations in response to acetylcholine, substance P and thrombin are impaired in various arteries from rabbits (Jayakody et al., 1985; Verbeuren et al., 1986), pigs (Hayashi et al., 1991) and monkeys (Freiman et al., 1986). In hypertension, endothelium-dependent relaxations in response to acetylcholine are impaired in aortas from experimental hypertensive rats (Van de Voorde and Leusen, 1986), spontaneously hypertensive rats (SHR) (Konishi and Su, 1983) and Dahl salt-sensitive (DS) rats (Lüscher et al., 1987). This impairment is considered to result from a simultaneous release of endothelium-derived contracting factor (EDCF) in addition to a normal release of endothelium-derived relaxing factor (EDRF) in SHR (Lüscher and Vanhoutte, 1986; Vanhoutte, 1989), whereas in the case of DS rats, it seems to occur due to a decreased release of EDRF (Lüscher et al., 1987; Vanhoutte, 1989). Thus, the association of hypercholesterolemia with hypertension is likely to result in progressive dysfunction of vascular endothelial cells, which may be of importance in initiating atherosclerosis. However, there has been little work in this area (Yu et al., 1993). In the present study, we investigated the combined effects of hypercholesterolemia and hypertension on endothelium-dependent and endothelium-independent relaxations in aortic rings from DS rats fed on a high-cholesterol and high-sodium diet.

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2. Materials and methods

2.1. Experimental animals

Male 4-week-old Dahl salt-sensitive (DS) rats (Eisai Co., Tokyo, Japan) were used. The rats were housed in an air-conditioned room (23 ± 1°C and 60 ± 10% humidity) under an artificial 12-h light/dark cycle (7:00 a.m.–7:00 p.m.) and given food and water ad libitum. The animals received a basal diet supplemented with 0.4% NaCl (control group, $n = 5$) or 8% NaCl (NaCl group, $n = 5$), or a high-cholesterol diet supplemented with 0.4% NaCl (CHOL group, $n = 5$) or 8% NaCl (NaCl + CHOL group, $n = 5$) for 8 weeks. The synthetic basal diet contained 20% casein, 63.2% sucrose, 10% corn oil, 2% agar, 0.8% vitamin mixture and 4% salt mixture (Fukushima et al., 1968), from which sodium chloride had been omitted. The high-cholesterol diet consisted of the basal diet with 1.5% cholesterol and 0.5% cholic acid. The systolic blood pressure was determined in conscious rats by the indirect tail-cuff method once a week.

2.2. Aortic preparations

Rats were anesthetized with pentobarbital sodium (40 mg/kg, i.p.) after overnight fasting and killed by bleeding from a cannula inserted into the abdominal aorta, and thoracic aortas were excised and immediately placed into the Krebs Henseleit solution (118.4 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25.0 mM $NaHCO_3$, 11.1 mM glucose). The blood was centrifuged at 3000 r.p.m. for 10 min and the serum was preserved at −40°C for lipid analysis. The thoracic aortas were cleaned of adhering connective tissue, and ring segments 2 mm wide were prepared from each vessel. Great care was taken to preserve the endothelium. Each ring was fixed vertically under a resting tension of 1.0 g in a 10-ml organ bath filled with the solution (37°C, pH 7.4) described above, which was continuously aerated with a gas mixture of 95% O_2 and 5% CO_2 and then was allowed to equilibrate for 90 min before the start of the experiment. Isometric tension change was measured with a force-displacement transducer (Model t-7, NEC San-Ei, Tokyo, Japan) coupled to an ink-writing oscillograph (Model 8K21, NEC San-Ei).

2.3. Vascular reactivity

The rings with intact endothelium were contracted twice with noradrenaline (10^{-7} M). When the second response to noradrenaline reached a plateau, causing approx. 80% of the maximal contractile response, as estimated in preliminary experiments, acetylcholine (10^{-9}–10^{-5} M), calcium ionophore A23187 (10^{-9} M–10^{-6} M) and sodium nitroprusside (10^{-10}–10^{-5} M) were cumulatively added to the bath solution. The relaxation response was expressed as a percentage of the maximal relaxation developed in response to papaverine (10^{-4} M). EC_{50} values for vasodilator responses were obtained from individual concentration-response curves as the concentration at which half-maximal reduction in recipient tone occurred. The absolute level of tension induced by noradrenaline (10^{-7} M) did not differ statistically between the different groups.

2.4. Lipid analysis

The aortic ring segments used in vascular reactivity experiments were used to measure cholesterol. The rings were freeze-dried, and their lipids were extracted at 50°C for 20 min with chloroform-methanol (2:1, v/v). The cholesterol content of the lipid extract together with the serum was determined by the fluoroenzymatical methods described previously by Kunitomo et al. (1984).

2.5. Drugs

Drugs used in the present experiments were as follows: acetylcholine chloride (Daiichi Pharmaceutical Co., Tokyo, Japan); calcium ionophore A23187 (Sigma Chemical Co., St. Louis, MO, USA); papaverine hydrochloride and sodium nitroprusside (Nacalai Tesque,

Table 1
Blood pressure, serum cholesterol levels and aortic cholesterol contents in Dahl salt-sensitive rats

	Number of animals	Systolic blood pressure (mmHg)	Serum total cholesterol (mg/100 ml)	Aortic cholesteryl ester (mg/g dry weight)
Control	5	145 ± 1	67 ± 2	0.645 ± 0.048
CHOL	5	149 ± 1	297 ± 28 *,#	0.631 ± 0.055
NaCl	5	193 ± 3 *	73 ± 3	0.678 ± 0.032
NaCl + CHOL	5	218 ± 6 *,#	377 ± 13 *,#	1.149 ± 0.075 *,#

Dahl salt-sensitive rats were divided into four groups. For 8 weeks, animals were fed on a low-sodium diet (control group), a high-cholesterol diet (CHOL group), a high-sodium diet (NaCl group) or a high-sodium plus high-cholesterol diet (NaCl + CHOL group). Each value represents the means ± S.E.M. * $P < 0.01$, as compared with the control group. # $P < 0.01$, as compared with the respective basal diet group (NaCl or control groups).

Kyoto, Japan); noradrenaline (Sankyo Co., Tokyo, Japan).

2.6. Statistical analysis

The data were expressed as means ± S.E.M. In each protocol, the number of rings studied was equal to the number of rats used. Statistical analysis was performed by ANOVA, followed by Scheffé's F-test. A difference was considered statistically significant when $P < 0.05$.

3. Results

3.1. Blood pressure

The systolic blood pressure of rats rapidly increased on sodium feeding. After 8 weeks on the diet supplied, the blood pressure significantly increased in the NaCl and NaCl + CHOL groups compared to the control group ($P < 0.01$, respectively), and the value in the NaCl + CHOL group was significantly greater than that in the NaCl group ($P < 0.01$). The blood pressure in the CHOL group was at the same level as that in the control group (Table 1). There was no significant difference in the body weights of the four groups.

3.2. Serum cholesterol levels and aortic cholesteryl ester content

Table 1 shows the serum total cholesterol levels and aortic cholesteryl ester contents in the four groups at the end of the experiment. The serum total cholesterol levels in the CHOL and NaCl + CHOL groups fed on a high-cholesterol diet were significantly higher than those in the control and NaCl groups ($P < 0.01$), respectively, and the level in the NaCl + CHOL group was significantly higher than that in the CHOL group ($P < 0.05$). A significant increase in the aortic content of cholesteryl ester, the major cholesterol accumulated, was observed only in the NaCl + CHOL group when compared with the other three groups ($P < 0.01$).

3.3. Acetylcholine- and A23187-induced relaxation responses

Endothelium-dependent relaxations in response to acetylcholine were significantly attenuated in the NaCl and NaCl + CHOL groups compared to the control group, and the degree of attenuation of the relaxation responses was significantly greater in the NaCl + CHOL group than in the NaCl group ($P < 0.01$) (Fig. 1). The EC_{50} value for acetylcholine was significantly higher in the NaCl ($8.37 \pm 1.45 \times 10^{-8}$ M, $P < 0.05$) and NaCl + CHOL groups ($14.1 \pm 1.5 \times 10^{-8}$ M, $P < 0.01$) than in the control group ($3.06 \pm 0.44 \times 10^{-8}$ M).

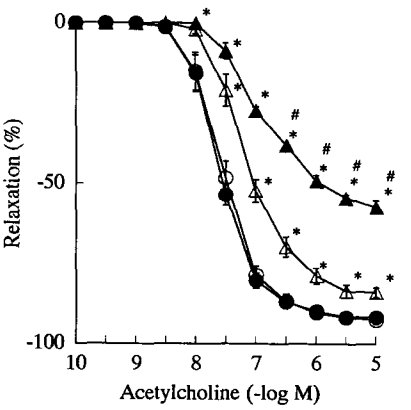

Fig. 1. Concentration-relaxation response curves for acetylcholine in endothelium-intact aortic rings isolated from Dahl salt-sensitive rats in control (○), CHOL (●), NaCl (△) and NaCl + CHOL (▲) groups. Aortic rings were preconstricted with 10^{-7} M noradrenaline. Relaxation was expressed as a percentage of that induced by 10^{-4} M papaverine. Each point represents the mean ± S.E.M ($n = 5$). * $P < 0.05$, as compared with the control group, # $P < 0.05$, as compared with the respective basal diet group (NaCl or control groups).

The maximal relaxation responses to acetylcholine in the NaCl and NaCl + CHOL groups were 87.2% ($P < 0.01$) and 54.4% ($P < 0.01$) of the control, respectively. Endothelium-dependent relaxations in response to A23187 also were significantly attenuated in the NaCl + CHOL group but not in the NaCl group compared to the control group. The maximal relaxation response to A23187 in the NaCl + CHOL group was 69.2% of the control ($P < 0.05$) (Fig. 2).

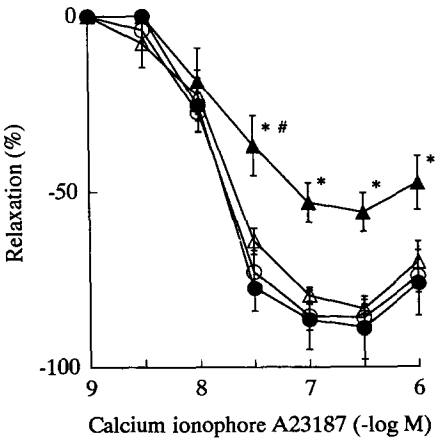

Fig. 2. Concentration-relaxation response curves for the calcium ionophore A23187 in endothelium-intact aortic rings isolated from Dahl salt-sensitive rats in control (○), CHOL (●), NaCl (△) and NaCl + CHOL (▲) groups. Aortic rings were preconstricted with 10^{-7} M noradrenaline. Relaxation was expressed as a percentage of that induced by 10^{-4} M papaverine. Each point represents the mean ± S.E.M ($n = 5$). * $P < 0.05$, as compared with the control group, # $P < 0.05$, as compared with the respective basal diet group (NaCl or control groups).

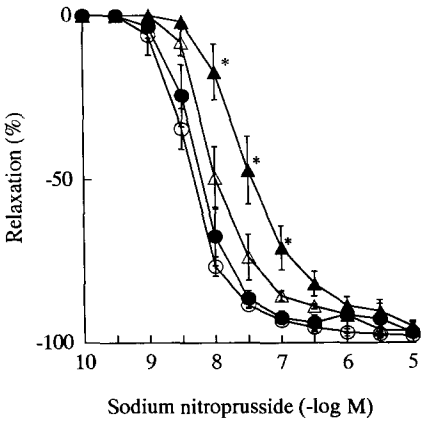

Fig. 3. Concentration-relaxation response curves for sodium nitroprusside in endothelium-intact aortic rings isolated from Dahl salt-sensitive rats in control (○), CHOL (●), NaCl (△) and NaCl + CHOL (▲) groups. Aortic rings were preconstricted with 10^{-7} M noradrenaline. Relaxation was expressed as a percentage of that induced by 10^{-4} M papaverine. Each point represents the mean ± S.E.M ($n = 5$). * $P < 0.05$, as compared with the control group.

3.4. Sodium nitroprusside-induced relaxation responses

Endothelium-independent relaxations in response to sodium nitroprusside were slightly but significantly attenuated in the NaCl + CHOL group compared to the control group (Fig. 3). The EC_{50} value for sodium nitroprusside in the NaCl + CHOL group was significantly higher than that in the control group ($23.6 \pm 5.1 \times 10^{-9}$ M vs. $4.40 \pm 0.68 \times 10^{-9}$ M, $P < 0.05$). However, the maximal relaxation responses to sodium nitroprusside were not modified in the four groups. The endothelium-dependent and endothelium-independent relaxations observed in the CHOL group were comparable to those in the control group.

4. Discussion

Dahl salt-sensitive (DS) rats are very similar to a subgroup of humans with hypertension in whom sodium contributes to the development of high blood pressure (Dahl et al., 1968; Nicholls, 1984). In the aorta of DS rats fed on a high-sodium diet, endothelium-dependent relaxations in response to various vasodilators are significantly impaired, while the relaxations in response to the endothelium-independent agonist, sodium nitroprusside, are only slightly impaired (Lüscher et al., 1987; Boegehold, 1992). In the present study, we confirmed similar changes in vascular responses of the aorta of hypertensive DS rats, and further demonstrated that cholesterol feeding can markedly enhance the impaired endothelium-dependent relaxations in response to acetylcholine and A23187, together with endothelium-independent relaxations in response to sodium nitroprusside. Also, we found that cholesterol feeding, in addition to further elevating blood pressure, causes a great increase in the serum cholesterol level in these DS rats, and consequently an appreciable cholesteryl ester deposition in the aorta. These results indicate that high-cholesterol intake in rats with salt-induced hypertension aggravates the functional changes predominantly in arterial endothelial cells and moderately in smooth muscle cells, although it is not known whether these impaired functions are the cause or the consequence of the exacerbation of hypertension and aortic cholesterol deposition. Verbeuren et al. (1986, 1990) have reported that in severely atherosclerotic arteries of rabbits, not only the endothelium-dependent relaxations in response to acetylcholine, but also the endothelium-independent relaxations in response to nitroglycerin are attenuated, and concluded that smooth muscle cell responsiveness to vasodilator substances may become affected as atherosclerosis progresses. Thus, in hypertensive DS rats fed on a high-cholesterol diet, the impaired functions of smooth muscle cells also may be related to aortic cholesterol deposition.

There are a number of reports describing the impairment of endothelium-dependent relaxation in rabbits (Jayakody et al., 1985; Verbeuren et al., 1986), pigs (Hayashi et al., 1991) or monkeys (Freiman et al., 1986) with hypercholesterolemia. In the aorta of normotensive DS rats, however, cholesterol feeding did not affect endothelium-dependent or endothelium-independent relaxations. Similar findings have been observed with Sprague-Dawley rats (Kitagawa et al., 1992). In contrast, Yu et al. (1993) have reported that in the aorta of SHR as well as normotensive Wistar Kyoto rats, cholesterol feeding progressively impairs the endothelium-dependent relaxations in response to acetylcholine but does not modify the endothelium-independent relaxations in response to sodium nitroprusside. This shows that hypercholesterolemia can interfere with endothelium function independently of hypertension. Thus, it seems that the susceptibility of the endothelium to hypercholesterolemia differs greatly among rat strains. Additional work is needed to clarify the mechanism of this strain difference.

According to the present study, cholesterol feeding did not affect the aortic cholesterol content in DS rats on a low-sodium diet. Generally, rats are resistant to the development of atherosclerosis and therefore require injurious stimuli of the arterial wall in addition to feeding with a high-cholesterol diet (Kitagawa et al., 1992). Even in the SHR, in which subendothelial thickening in the arterial wall develops with increasing blood pressure (Limas et al., 1980), cholesterol deposition in the aorta is not precipitated by cholesterol feeding (Yamaguchi et al., 1995). Mori et al. (1993)

have shown that aortic cholesterol deposition is independent of increased blood pressure in SHR and stroke-prone SHR. In hypertensive DS rats, unlike in the case of SHR, appreciable cholesteryl ester accumulation occurs in the aorta, which is known to exhibit pathologic lesions in the intima (Limas et al., 1982). The differences in the morphological changes in the aortas of different strains of hypertensive rats also remain unknown. Since the main difference in these hypertensive rats is the treatment with a high-sodium diet, high-sodium intake might play a very important role in the injury to the endothelium.

Let us consider some possible explanations for the differences in the effects of cholesterol feeding on the vascular function and morphology of the two types of genetic hypertensive rats – SHR and DS rats. First, the release of endothelium-derived factors may be different. The impairment of endothelium-dependent relaxation in the SHR is not due to a decreased release of EDRF but to an increased release of EDCF (Lüscher and Vanhoutte, 1986; Kato et al., 1990), whereas that in the DS rat reflects a decreased release of EDRF itself (Lüscher et al., 1987). EDRF inhibits platelet adherence and aggregation, smooth muscle proliferation, and endothelial cell-leukocyte interactions, all of which are key events in atherosclerosis (Hogan et al., 1988; Kubes et al., 1991; Scott-Burden et al., 1992). Therefore, the reduction of EDRF activity observed in hypertensive DS rats may be an important endothelial alteration contributing to the atherogenic process, as recognized in hypercholesterolemic rabbits. Secondly, high-sodium intake may directly injure the endothelium independent of an influence on increased blood pressure. It has been shown that in the DS rat, the extent and severity of subendothelial space expansion correlates more closely with the duration of salt feeding than with the level of blood pressure (Limas et al., 1982). Likewise, in the SHR, high-sodium intake can cause unique vascular lesions such as fibrin deposition, but such depositions are rarely seen even in the SHR with severe hypertension (Limas et al., 1980). Moreover, hypertensive DS rats but not SHR exhibit glomerular injury with hyperlipidemia (O'Donnell et al., 1989). Such hyperlipidemia is markedly magnified when the rats are given a high-cholesterol diet (Kunitomo et al., 1985). These possibilities may account for the enhanced impairment of the vasodilator response and exclusive cholesterol deposition in the aorta of DS rats fed on a high-sodium plus high-cholesterol diet.

In summary, the present study demonstrates that cholesterol feeding enhances the impaired endothelium-dependent and -independent relaxations in aortas of hypertensive Dahl rats, parallel to the development of hypertension, hypercholesterolemia and cholesteryl ester deposition. These findings suggest that hypercholesterolemia together with salt-dependent hypertension may potentiate injury to the arterial wall, predominantly the endothelium, and accelerate the development of atherosclerosis. Excessive cholesterol intake by patients with salt-dependent hypertension may seriously aggravate cardiovascular complications.

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