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Characterization of endothelium-dependent relaxation and modulation by treatment with pioglitazone in the hypercholesterolemic rabbit renal artery

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Abstract

The present study was undertaken to investigate vascular function in hypercholesterolemic rabbits and also to characterize the effects of pioglitazone on it. Rabbits were fed normal, 0.5% cholesterol chow, or 0.5% cholesterol chow plus 300 ppm pioglitazone for 5 or 10 weeks. The tension of isolated renal artery rings was measured isometrically, and morphometric analysis was performed. The cholesterol chow diet administered for 5 weeks did not affect acetylcholine-induced relaxation in the renal artery but that for 10 weeks decreased it. The N^G-nitro-L-arginine (L-NOARG)- and indomethacin-resistant endothelium-dependent relaxation induced by acetylcholine in the renal artery was enhanced in rabbits receiving the cholesterol chow for 5 or 10 weeks, as compared to rabbits receiving the control diet, and the percentage of plaque area formation was increased in the renal artery by the cholesterol chow for 10 weeks. Pioglitazone normalized them without lowering serum lipid levels. The resistant parts of acetylcholine-induced relaxation was significantly inhibited when the renal artery was treated with charybdotoxin, an inhibitor of large and intermediate conductance Ca^{2+}-activated K^+ channels, or *N*,*N*-diethylaminoethyl-2,2-diphenylvalerate hydrochloride (SKF 525a), a cytochrome P-450 monooxygenase inhibitor. Results indicate that hypercholesterolemia enhances endothelium-derived hyperpolarizing factor (EDHF)-mediated relaxation in the rabbit renal artery and pioglitazon normalizes it without lowering serum lipid levels, and suggest that the maintenance of endothelial function by pioglitazon is related to the mechanisms for its anti-atheromatous activity.

Keywords: Hypercholesterolemia; Renal artery; Endothelium-derived hyperpolarizing factor; Pioglitazone

1. Introduction

Several reports have shown that endothelium-dependent relaxation is regulated by the release of mainly three different relaxing factors in the endothelium: the cyclo-oxygenase metabolite prostacyclin (Moncada et al., 1977), the endothelial nitric oxide (NO) synthase product NO (Moncada and Higgs, 1993), and endothelium-derived hyperpolarizing factor (EDHF) (Mommbouli and Vanhotte, 1997). EDHF is thought to be K^+ (Edwards et al., 1998), a cannabinoid (Randall et al., 1996), or a cytochrome P-450 monooxygenase-arachidonic acid metabolite (Taylor and Weston, 1988), which has been proposed to belong to the group of epoxyeicosatrienoic acids (Hecker et al., 1994; Campbell et al., 1996). EDHF may be important in the vasorelaxation induced by acetylcholine and is resistant to N^G-nitro-L-arginine (L-NOARG), an endothelial NO synthase (NOS) inhibitor (Ishii et al., 1990) and indomethacin, a cyclooxygenase inhibitor (Ferreira et al., 1971). The endothelium-dependent hyperpolarization is due to the opening of Ca^{2+}-activated K^+ channels (Cohen and Vanhoutte, 1995; Garland et al., 1995; Chen and Suzuki, 1989).

Pioglitazone, a novel oral antihyperglycemic agent classified as a thiazolidinedione, increases insulin sensitivity and responsiveness of target tissues (Ikeda et al., 1990).

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Pioglitazone may also be effective in treating intimal hyperplasia in the rat aorta after balloon (Law et al., 1996) and for treatment of hypertension (Kotchen et al., 1996). Endothelial dysfunction is a feature of these chronic disorders. Hypercholesterolemia is also associated with impairment of endothelium-dependent relaxation in rabbits (Chappell et al., 1987; Jayakody et al., 1987; Verbeuren et al., 1990) and humans (Ludmer et al., 1986). Impairment of endothelium-dependent relaxation may partially result in NO inactivation by oxygen-derived free radicals (Adeagbo and Triggle, 1993; Ohara et al., 1993). We previously observed that in rabbit aorta, endothelial NO-dependent relaxation induced by acetylcholine was decreased and the percentage of plaque area formation was increased by the cholesterol chow after 5 and 10 weeks of diet administration, and also observed that pioglitazone normalized both the NO-dependent relaxation and the plaque formation without lowering serum lipid levels (unpublished observation). Pioglitazone may influence on NO system in endothelium, but the detail mode of action is not clear. Further, the effects of pioglitazone on EDHF-mediated vasorelaxation in hypercholesterolemia is unknown yet. The influence of cholesterol-containing diets on EDHF-mediated vasorelaxation has been evaluated in only a few reports (Najibi and Cohen, 1995; Brandes et al., 1997; Honda et al., 2001), and the detailed relationship between the NO- and EDHF-mediated vasorelaxation in hyperchoresterolemia still remains unclear. Thus, the present study was undertaken to investigate the alteration in vascular function, especially EDHF-mediated vasorelaxation, in the hyperchoresterolemic rabbit renal artery and to study the effect of pioglitazone on it.

2. Material and methods

2.1. Animals and tissues

All procedures were performed in accordance with institutional guidelines for animal research at Tokyo University of Pharmacy and Life Science. Ten-week-old male Jla:JW rabbits supplied by Japan Laboratory Animal (Tokyo, Japan) were used. Rabbits were fed normal chow, 0.5% cholesterol chow, or 300 ppm pioglitazone with 0.5% cholesterol chow (Funabashi Nojou, Chiba, Japan) for 5 or 10 weeks. The animals were anesthetized with pentobarbital (40 mg/kg, i.v.) and sacrificed by bleeding. The renal artery was isolated and placed in modified Krebs–Henseleit solution having the composition (mM): NaCl, 118.0; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; glucose, 11.0 at 37 °C gassed with 95% O_2 and 5% CO_2. The tissues were cleaned by removing connective tissue. The renal artery was cut into rings about 4 mm long. Contraction was measured by suspending the rings between two stainless-steel hooks, one of which was attached to the end of a bathing tube and the other connected to a force transducer (SB-1T Nihonkoden, Japan) (Unemoto et al., 2003).

2.2. Relaxation of arteries pre-contracted by phenylephrine

Each preparation was equilibrated in 10 ml of bathing solution for 60–90 min before the experiment. The resting tension was 1.5 g, which was the optimal preload for force development in these vessels determined in preliminary studies (Honda et al., 2001). Isometric tension changes were recorded on a polygraph (RECTI-HORIZ-8K San-ei, Japan). After equilibration the rings were exposed to KCl (50 mM). When the contractile responses reached plateaus, the rings were rinsed with modified Krebs–Henseleit solution and allowed to equilibrate for an additional 60 min before the application of phenylephrine (3×10^{-7} M). For the relaxation studies, the submaximal tone was induced with phenylephrine (3×10^{-7} M), and then acetylcholine (10^{-9}–3×10^{-6} M) or sodium nitroprusside (10^{-11}–3×10^{-6} M) was added (Ampong et al., 2002).

2.3. Effects of potassium channel blockers and a cytochrome P-450 monooxygenase inhibitor on L-NOARG- and indomethacin-resistant relaxation induced by acetylcholine

The effects of ATP-sensitive, voltage-dependent and Ca^{2+}-activated K^+ channels or cytochrome P-450 monooxygenase activity on acetylcholine-induced relaxation

Table 1
Body weight and serum lipid levels after treatment with cholesterol diet for 5 or 10 weeks

Groups	BW (kg)	TC (mg/dl)	TG (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)
5 weeks					
Control	3.01±0.18	33±3	63±35	13±1	17±3
Cholesterol	3.04±0.10	1697±215[a]	37±5	538±61[a]	58±7[a]
Pioglitazone	2.71±0.02	2064±130[a]	48±7	698±53[a]	77±5[a]
10 weeks					
Control	3.52±0.07	23±2	28±7	8±1	12±1
Cholesterol	3.50±0.17	1733±312[a]	92±45	648±85[a]	68±5[a]
Pioglitazone	3.27±0.07	1754±188[a]	36±8	627±80[a]	48±9[a]

TC: total cholesterol, TG: triglyceride, LDL-C: low density lipoprotein cholesterol, HDL-C: high density lipoprotein cholesterol. Each value is the mean±S.E.M. from six rabbits.

[a] $P<0.01$ from control. BW: body weight.

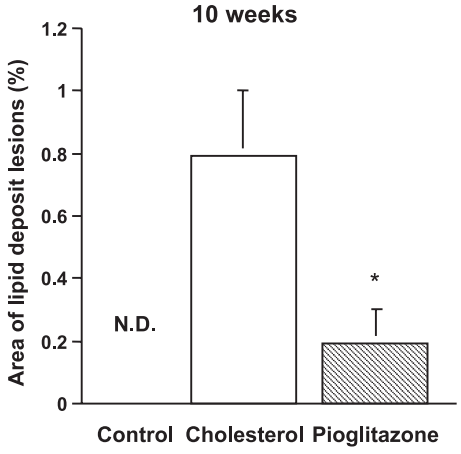

Fig. 1. Comparison of ratio of the lipid deposition area on the tunica intima of the renal artery in the hypercholesterolemic rabbit. Each column represents mean±S.E.M. from six rabbits. ND: Not determined. *P<0.05 from cholesterol.

were evaluated in the renal artery. L-NOARG (10^{-4} M), a NOS inhibitor (Ishii et al., 1990), indomethacin (10^{-5} M), a prostaglandin synthase inhibitor (Ferreira et al., 1971), glibenclamide (3×10^{-6} M), a selective inhibitor of ATP-sensitive K^+ channels (Standen et al., 1989), 4-aminopyridine (10^{-3} M), a selective inhibitor of voltage-dependent K^+ channels (Honda et al., 1999), tetraethylammonium (TEA, 10^{-3} M), a non-selective inhibitor of Ca^{2+}-activated K^+ channels (Holland et al., 1996), charybdotoxin (10^{-7} M), an inhibitor of large and intermediate conductance Ca^{2+}-activated K^+ channels (Garcia et al., 1995), or *N*,*N*-diethylaminoethyl-2,2-diphenylvalerate hydrochloride (SKF 525a) (10^{-5} M), an inhibitor of cytochrome P-450 monooxygenase enzymes (Campbell et al., 1996) were added to the solution 10 min before treatment with phenylephrine. To assess the role of the endothelium in relaxation resistance to acetylcholine, some renal arteries were de-endothelialized by gentle rubbing of the luminal surface with a string before they were mounted.

2.4. Determination of serum lipid

Blood samples were collected from each rabbit before excision of the arteries. After centrifugation at 1200×g for 15 min, serum total cholesterol and triglyceride levels

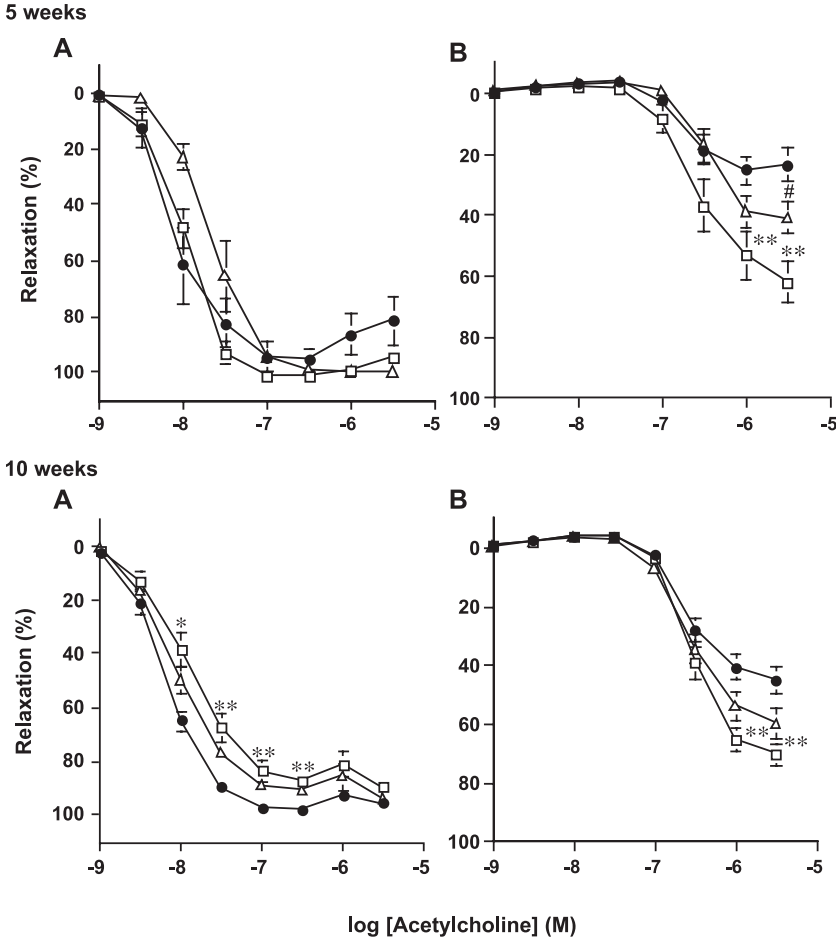

Fig. 2. Acetylcholine-induced relaxation of renal artery rings pre-contracted with phenylepohrine in the absence (A) or the presence (B) of L-NOARG (10^{-4} M) and INDO (10^{-5} M) in the hypercholesterolemic rabbit. Rabbits were fed control, cholesterol, or cholesterol+pioglitazone diets for 5 or 10 weeks. L-NOARG: N^G-nitro-L-arginine. INDO: Indomethacin. Each value is the mean±S.E.M. from six rabbits. *P<0.05, **P<0.01 from control. #P<0.05 from cholesterol.

were measured by a standard enzymatic method. High density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) were measured by the heparin–nickel method and heparin–citric acid method, respectively.

2.5. Histological preparation

After the final blood collection, the arteries were excised for histological study. The arteries were stored in Bouin solution. Paraffin-embedded specimens were stained with haematoxylin–eosin–safran, sectioned at 4 μm (30 sections in succession) and examined by light microscopy to measure fatty streak. The score of fatty streak was determined as the area of lipid deposit and it was divided by the area of intima and media to achieve the percentage of lipid deposit lesions.

2.6. Drugs and chemicals

Phenylephrine, acetylcholine, L-NOARG, 4-aminopyridine, TEA, charybdotoxin, or SKF 525a (Sigma, St. Louis, MO, USA) was dissolved in distilled water. Glibenclamide (Sigma) was dissolved in ethanol (the final concentration less than 0.5% ethanol in the bath did not influence PE-induced contraction). Indomethacin was dissolved in 4% (w/v) $NaHCO_3$. The other chemicals were of analytical grade and obtained from Wako (Osaka, Japan).

2.7. Statistical analysis

Values were expressed or plotted as mean±S.E.M. Two-way analysis of variance (ANOVA) with a post-ANOVA test was used to compare the concentration–response curves. Individual points were compared using the Student's *t*-test or the multiple Dunnett test, and differences were considered significant at $P<0.05$.

3. Results

3.1. Serum lipid levels

Body weights did not differ significantly between the control, cholesterol and pioglitazone groups after 5 or 10 weeks of treatment. Administration of the high cholesterol diet for 5 or 10 weeks significantly elevated the serum total cholesterol, LDL-C, and HDL-C levels, while pioglitazone treatment influenced these serum lipid levels very little. Serum triglyceride levels were not markedly altered by either the high cholesterol diet or pioglitazone treatment (Table 1).

3.2. Plaque area

Administration of the cholesterol diet for 5 weeks had no influence on intimal plaque formation in the renal artery. Administration of the cholesterol diet for 10 weeks caused significant intimal plaque formation and treatment with pioglitazone significantly suppressed it (Fig. 1).

3.3. Relaxation of the renal artery pre-contracted by phenylephrine

The tension induced by KCl (50 mM) was 4.39±0.49, 5.41±0.34, and 5.68±0.32 g in the control, cholesterol-fed, and pioglitazone plus cholesterol-fed rabbits for 5 weeks, respectively. The tension induced by phenylephrine (3×10^{-7} M) was 2.63±0.64, 2.84±0.31, and 2.11±0.13 g in the control, cholesterol-fed, and pioglitazone plus cholesterol-fed rabbits for 5 weeks, respectively. Contractions did not differ among the three groups. The tension induced by KCl (50 mM) was 5.18±0.36, 5.24±0.25, and 5.25±0.25 g in the control, cholesterol-fed, and pioglitazone plus cholesterol-fed rabbits for 10 weeks, respectively. And the tension induced by phenylephrine (3×10^{-7} M) was 3.42±0.81, 2.45±0.53, and 2.51±0.42 g in the control, cholesterol-fed, and pioglitazone plus cholesterol-fed rabbits for 10 weeks, respectively. Contractions did not differ among these three groups either.

Addition of acetylcholine (10^{-9}–3×10^{-6} M) produced dose-dependent relaxation in the renal artery rings pre-contracted by PE from all rabbits (Fig. 2A). Acetylcholine-induced relaxation of the renal artery did not differ among the control, cholesterol-fed, and pioglitazone

Table 2
The negative logarithm of ED_{50} values for efficacy and maximal relaxation induced by acetylcholine in isolated renal artery

	Groups	−log (ED_{50}, M)	Max. relaxation (%)
5 weeks			
Acetylcholine	Control	7.55±0.40	95.6±4.0
	Cholesterol	7.97±0.08	101.1±0.8
	Pioglitazone	7.78±0.29	100.0±0.1
+L-NOARG+INDO	Control	6.77±0.18	27.5±4.6
	Cholesterol	6.55±0.10	62.0±7.0[a]
	Pioglitazone	6.37±0.05	42.3±5.3[b]
10 weeks			
Acetylcholine	Control	8.17±0.06	99.8±0.6
	Cholesterol	7.87±0.12	91.0±2.5
	Pioglitazone	7.75±0.15	97.0±1.1
+L-NOARG+INDO	Control	6.64±0.68	47.6±4.3
	Cholesterol	6.65±0.05	71.0±3.8[a]
	Pioglitazone	6.66±0.09	61.0±5.1[b]

ED_{50} values for efficacy were defined as percentage of relaxation per dose of agonist divided by maximal relaxation achieved in the arterial rings. Rabbits were fed control, cholesterol or cholesterol+pioglitazone diet for 5 or 10 weeks. L-NOARG: N^G-nitro-L-arginine. INDO: Indomethacin. Each value is the mean±S.E.M. from 4–6 rabbits.

[a] $P<0.01$ from control.

[b] $P<0.05$ from cholesterol.

plus cholesterol-fed rabbits after administration of the diets for 5 weeks (Fig. 2A, Table 2). However, administration of the high cholesterol chow for 10 weeks significantly impaired acetylcholine-induced relaxation of the renal artery and treatment with pioglitazone somewhat restored the impairment. Addition of sodium nitroprusside (10^{-11}–3×10^{-6} M) produced dose-dependent relaxation in the renal artery rings pre-contracted by phenylephrine from all rabbits, and sodium nitroprusside-induced vasorelaxation did not differ among the control, cholesterol-fed, and pioglitazone plus cholesterol-fed rabbits in the 5- and 10-week groups (data not shown).

Vasorelaxation induced by acetylcholine was significantly suppressed in the presence of L-NOARG (10^{-4} M) and indomethacin (10^{-5} M) in the 5- and 10-week groups (Fig. 2B, Table 2). L-NOARG- and indomethacin-resistant relaxation induced by acetylcholine was significantly enhanced in the rings from cholesterol-fed rabbits as compared to control rabbits in both the 5- and 10-week groups. Pioglitazone partially normalized the enhancement.

3.4. Effects of K^+ channel blockers on L-NOARG- and indomethacin-resistant relaxation induced by acetylcholine in the renal artery

To examine the involvement of K^+ channels in L-NOARG- and indomethacin-resistant relaxation induced by acetylcholine, the effects of pretreatment with glibenclamide, 4-aminopyridine, TEA and charybdotoxin were investigated. Addition of glibenclamide (3×10^{-6} M) did not markedly influence the L-NOARG- and indomethacin-resistant relaxation induced by acetylcholine except in the 10-week cholesterol fed group (Fig. 3). The resistant part of acetylcholine-induced relaxation was significantly impaired after addition of 4-aminopyridine (10^{-3} M) to the arteries from the cholesterol or pioglitazone 5- and 10-week treated animals (Fig. 3). There were significant differences in dose–response curves between groups. The resistant part of acetylcholine-induced relaxation was almost abolished after addition of TEA (10^{-3} M) to the arteries from these groups (Fig. 4). TEA was most effective at impairing the resistant part of acetylcholine-induced relaxation. Moreover, charybdotoxin is more specific for large and intemediate

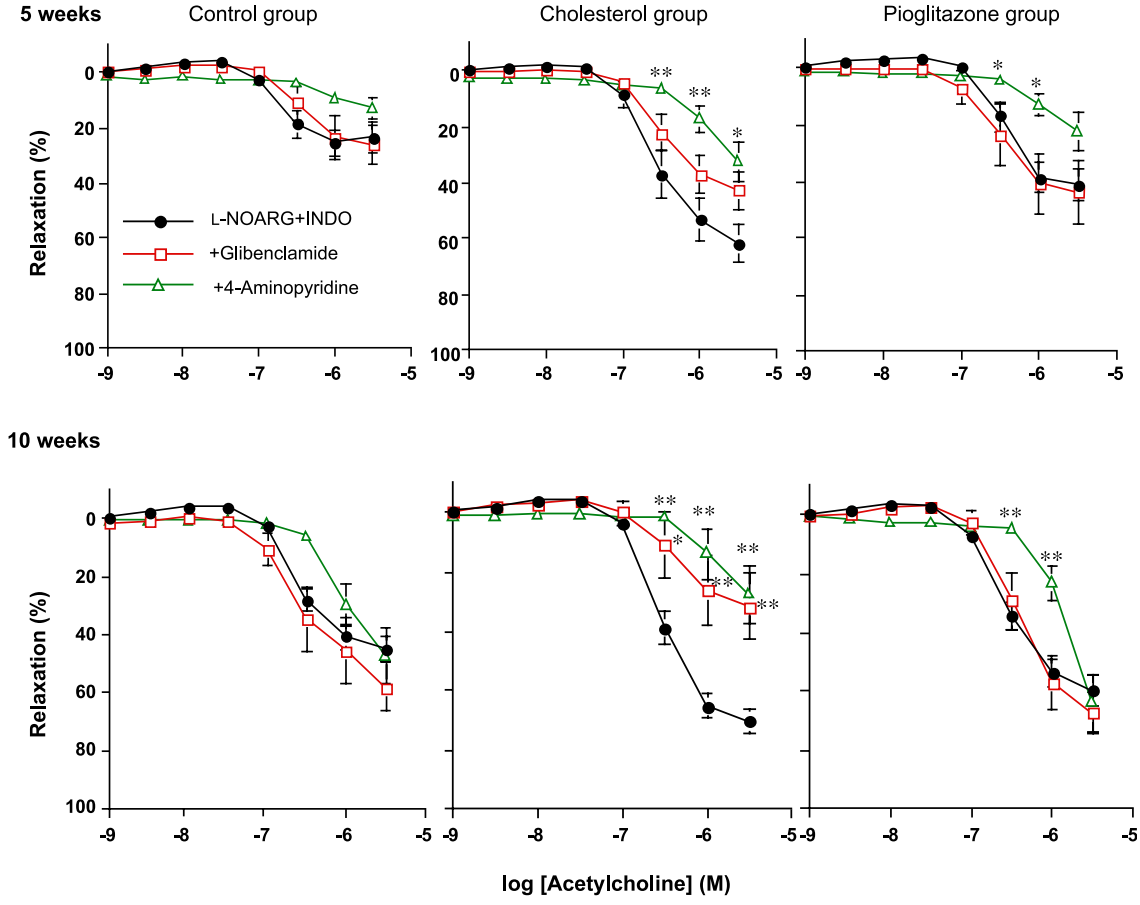

Fig. 3. Effects of glibenclamide (3×10^{-6} M) and 4-aminopyridine (10^{-3} M) on the relaxation of renal artery rings induced by acetylcholine in the presence of L-NOARG (10^{-4} M) and INDO (10^{-5} M). Rabbits were fed control, cholesterol, or cholesterol+pioglitazone diets for 5 or 10 weeks. L-NOARG: N^G-nitro-L-arginine. INDO: Indomethacin. Each value is the mean±S.E.M. from six rabbits. *P<0.05, **P<0.01 from L-NOARG+INDO.

conductance Ca^{2+}-activated K^+ channels (Garcia et al., 1995) almost abolished the resistant part of acetylcholine-induced relaxation in arteries from both the 5- and 10-week treatment groups and was nearly as effective as TEA and also significant differences were found in dose–response curves between groups (Fig. 4).

3.5. Effects of endothelial removal and a cytochrome P-450 monooxygenase inhibitor on L-NOARG- and indomethacin-resistant relaxation induced by acetylcholine in the renal artery

Next, to examine the involvement of the endothelium and cytochrome P-450 system in L-NOARG- and indomethacin-resistant relaxation induced by acetylcholine, the effects of endothelial removal and pretreatment with SKF 525a were investigated. Endothelial removal abolished the resistant part of acetylcholine-induced relaxation in the three groups fed for 5 or 10 weeks (Fig. 5). Significant differences in dose–response curves existed between groups. Addition of SKF 525a significantly inhibited the resistant part of acetylcholine-induced relaxation in the three groups fed for 5 or 10 weeks and also significant differences were seen in dose–response curves between groups.

4. Discussion

The present results have indicated that 5 weeks of cholesterol-feeding does not influence relaxation induced by acetylcholine in rabbit renal arteries, while 10 weeks of the diet significantly impairs relaxation induced by acetylcholine, and pioglitazone somewhat restores the impairment. Further, hypercholesterolemia significantly enhances the L-NOARG- and indomethacin-resistant relaxation to acetylcholine in both the 5- and 10-week feeding groups, and pioglitazone partially normalizes the enhancement. Interestingly, hypercholesterolemia in rabbit renal arteries induced by cholesterol feeding for 5 or 10 weeks enhanced the L-NOARG- and indomethacin-resistant relaxation to acetylcholine, though acetylcholine-induced vasorelaxation was not affected after 5 weeks of consuming the cholesterol diet but was inhibited after 10 weeks of consuming the diet. Enhanced resistant part of acetylcholine-induced vasorelaxation is probably not caused by altered contracting and relaxing arterial smooth muscle function because KCl- or phenylephrine-induced contraction and sodium nitroprusside-induced relaxation in the renal artery are not significantly affected by hypercholesterolemia. The combination of L-NOARG and indomethacin attenuate the response to

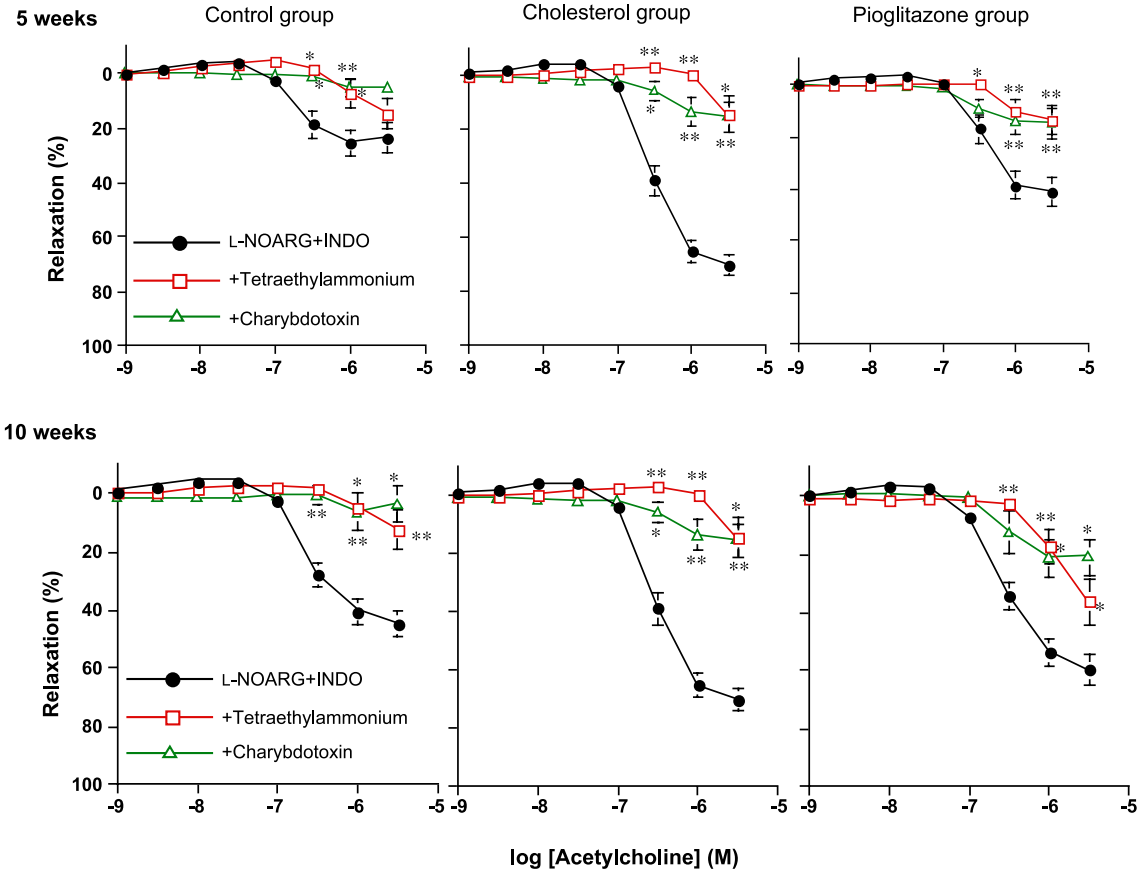

Fig. 4. Effects of tetraethylammonium (10^{-3} M) and charybdotoxin (10^{-7} M) on the relaxation of renal artery rings induced by acetylcholine in the presence of L-NOARG (10^{-4} M) and INDO (10^{-5} M). Rabbits were fed control, cholesterol, or cholesterol+pioglitazone diets for 5 or 10 weeks. L-NOARG: N^G-nitro-L-arginine. INDO: Indomethacin. Each value is the mean±S.E.M. from six rabbits. *P<0.05, **P<0.01 from L-NOARG+INDO.

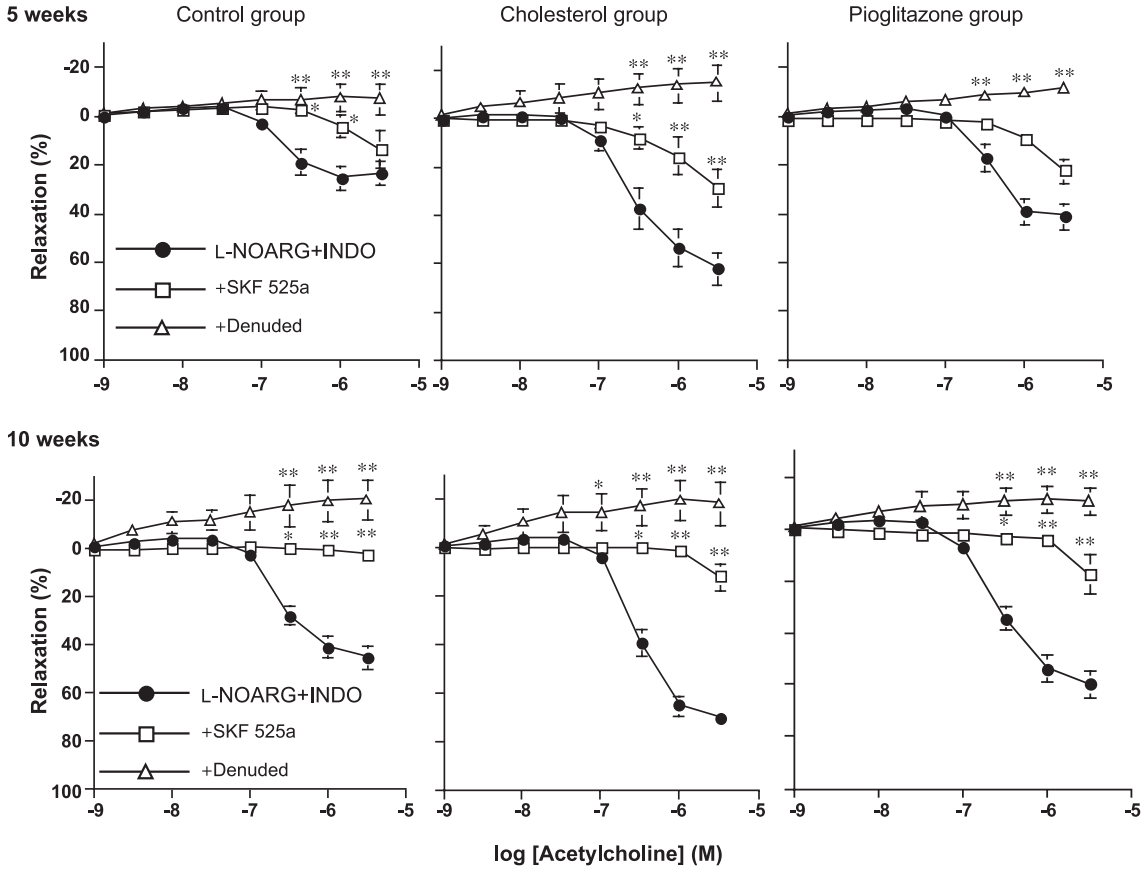

Fig. 5. Effects of SKF 525a (10^{-5} M) and endothelial denudation (Denuded) on the relaxation of renal artery rings induced by acetylcholine in the presence of L-NOARG (10^{-4} M) and INDO (10^{-5} M). Rabbits were fed control, cholesterol, or cholesterol+pioglitazone diets for 5 or 10 weeks. L-NOARG: N^G-nitro-L-arginine. INDO: Indomethacin. Each value is the mean±S.E.M. from six rabbits. *P<0.05, **P<0.01 from L-NOARG+INDO.

acetylcholine in control rabbits at 5 weeks to ~28% yet to only ~48% at 10 weeks. These findings have indicated that the age of the rabbit per se influences the relative contribution of NO and non-NO factors to endothelium-dependent relaxation and suggested that specifically non-NO factors appear to contribute to a greater extent as the animal ages.

L-NOARG- and indomethacin-resistant endothelium-dependent relaxation induced by acetylcholine was assumed to be mediated by EDHF. In our present study, the resistant part of acetylcholine-induced relaxation in the renal arteries was significantly inhibited when the artery was treated with TEA, a non-selective inhibitor of Ca^{2+}-activated K^+, or SKF 525a, a cytochrome P-450 monooxygenase inhibitor. This resistant part was completely abolished after denudation, suggesting its dependency on the presence of intact endothelial cell. These observations are in accordance with the studies about EDHF as it has been reported for other types of arteries from other species (Corriu et al., 1996; Kuhberger et al., 1994), including human coronary arteries (Nakashima et al., 1993). Furthermore, charybdotoxin, an inhibitor of large and intermediate conductance Ca^{2+}-activated K^+ channels, almost abolished the resistant part of acetylcholine-induced relaxation in the rabbit renal arteries. In the control rabbits, 4-aminopyridine, a selective inhibitor of voltage-dependent K^+ channels, appeared to attenuate in part the resistant components of the relaxation to acetylcholine. Thus, our results suggest that EDHF in the rabbit renal artery is cytochrome P-450 monooxygenase-arachidonic acid metabolites or related metabolites and it opens large and/or intermediate conductance Ca^{2+}-activated K^+ channels and possibly voltage-dependent K^+ channels. In contrast, following 10 weeks of hypercholesterolemia, glibenclamide, a selective inhibitor of ATP-sensitive K^+ channels, significantly attenuated the L-NOARG- and indomethacin-resistant relaxation to acetylcholine, suggesting that with hypercholesterolemia, ATP-sensitive K^+ channels are upregulated and mediate, together with Ca^{2+}-activated and voltage-dependent K^+ channels, the response to EDHF.

The interactions between NO and EDHF are not so clear. However, decreased levels of NO were reported to increase vasorelaxation mediated by EDHF in porcine coronary arteries (Bauersachs et al., 1996). Furthermore, the induction of inducible NOS by bacterial lipopolysaccharides suppressed EDHF-mediated relaxation in porcine coronary arteries (Kristof et al., 1997). Considering these reports, the enhancement of the EDHF component in the rabbit renal artery by hypercholesterolemia appears to be caused by attenuation of NO activity. In fact, hypercholesterolemia

increases oxygen-derived free radical production in the endothelium, which increases NO breakdown (Ohara et al., 1993; Mugge et al., 1994). The enhancement of the EDHF component may serve as a compensatory mechanism to maintain vasorelaxation in the disease states. In this study, EDHF-mediated relaxation was already increased at 5 weeks of cholesterol diet without the lipid deposit in the renal artery, suggesting that the dysfunction of NO system already appears in the endothelium before the histological alteration. Pioglitazone suppressed the enhancement of the EDHF component in the rabbit renal artery induced by hypercholesterolemia without lowering serum cholesterol levels. Pioglitazone may prevent NO degradation in the endothelium, since increased breakdown of NO by superoxide anions may contribute to the impairment of endothelium-dependent relaxation in hypercholesterolemia and arteriosclerosis (Ohara et al., 1993; Mugge et al., 1994). Indeed, we observed that hypercholesterolemic rabbit renal artery precontracted by KCl, pioglitazone prevented the impairment of endothelial NO-dependent relaxation induced by acetylcholine (unpublished observation). Peroxisome proliferator-activated receptor γ agonists such as pioglitazone and troglitazone were reported to increase CuZn-SOD gene expression and protein levels in endothelial cells of human umbilical vein and aorta (Inoue et al., 2001). CuZn-SOD is one of the superoxide scavenger enzymes. The increased levels of superoxide anions induced by hypercholesterolemia may enhance the alteration of NO to an inactive form. Pioglitazone may inhibit the inactivation of NO through the enhanced activity of CuZn-SOD to cause its anti-atherogenic actions. Pioglitazone may also decrease the contribution of ATP-sensitive K^+ channels to EDHF-mediated relaxation because glibenclamide no longer attenuated the EDHF relaxation in the 10-week cholesterol group and this may explain in part its ability to attenuate the compensatory relaxation by EDHF. The effects of pioglitazone in this study were not caused by toxicity, since symptoms of toxicity were not observed during the experimental period from the calculated dose administered from the diet of approximately 15 mg/kg/day pioglitazone for 5 weeks. Further, body weight changes were not significantly different in rabbits from the 5- or 10-week feeding groups or the control and pioglitazone groups. Chronic oral administration of 30 mg/kg/day pioglitazone for 13 weeks did not cause any toxic effects in rats (Maeshiba et al., 1997). Thus, the vasculo-protective effects of pioglitazone reported in this study are not due to toxicity.

In conclusion, the present results indicate that hypercholesterolemia enhances EDHF-mediated relaxation in the rabbit renal artery, and suggest that it serves as a compensatory mechanism to maintain relaxation in the disease states. Pioglitazone significantly restores this compensatory relaxation by EDHF, and it may be due to the inhibition of NO inactivation in the endothelium. Maintenance of endothelial function in hyperchoresterolemic rabbit renal artery by pioglitazone is apparently achieved through NO protection, which may be one of the mechanisms for its anti-atheromatous activity in the hypercholesterolemic rabbit artery.

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References

Adeagbo, A.S., Triggle, C.R., 1993. Varying extracellular [K^+]: a functional approach to separating EDHF- and EDNO-related mechanisms in perfused rat mesenteric arterial bed. J. Cardiovasc. Pharmacol. 21, 423–429.

Ampong, B., Honda, H., Kogo, H., 2002. Effect of hypothyroidism on beta-adrenoceptor-mediated relaxation in the rat thoracic aorta: a time-dependent study. Vasc. Pharmacol. 38, 149–155.

Bauersachs, J., Popp, R., Hecker, M., Sauer, E., Fleming, I., Busse, R., 1996. Nitric oxide attenuates the release of endothelium-derived-hyperpolarizing factor. Circulation 94, 3341–3347.

Brandes, R.P., Behra, A., Lebherz, C., Bîger, R.H., Bode-Bîger, S.M., Phivthong-Ngam, L., MÅgge, A., 1997. N^G-nitro-L-arginine- and indomethacin-resistant endothelium-dependent relaxation in the rabbit renal artery: effect of hypercholesterolemia. Atherosclerosis 135, 49–55.

Campbell, W.B., Gebremedhin, D., Pratt, P.F., Harder, D.R., 1996. Identification of epoxyeicosatrienoic acids as endothelium-derived hyperpolarizing factors. Circ. Res. 78, 415–423.

Chappell, S.P., Lewis, M.J., Henderson, A.H., 1987. Effect of lipid feeding on endothelium dependent relaxation in rabbit aortic preparations. Cardiovasc. Res. 21, 34–38.

Chen, G., Suzuki, H., 1989. Some electrical properties of the endothelium-dependent hyperpolarization recorded from rat arterial smooth muscle cells. J. Physiol. 410, 91–106.

Cohen, R.A., Vanhoutte, P.M., 1995. Endothelium-dependent hyperpolarization—beyond nitric oxide and cyclic GMP. Circulation 92, 3337–3349.

Corriu, C., Feletou, M., Canet, E., Vanhoutte, P.M., 1996. Inhibitors of the cytochrome P450-mono-oxygenase and endothelium-dependent hyperpolarization in the guinea-pig isolated carotid artery. Br. J. Pharmacol. 117, 607–610.

Edwards, G., Dora, K.A., Gardener, M.J., Garland, C.J., Weston, A.H., 1998. K^+ is an endothelium-derived hyperpolarizing factor in rat arteries. Nature 19, 269–272.

Ferreira, S.H., Moncada, S., Vaine, J.R., 1971. Indomethacin and aspirin abolish prostaglandin release from the spleen. Nat. New Biol. 231, 237–239.

Garcia, M.L., Knaus, H.G., Munujos, P., Slaughter, R.S., Kaczorowski, G.J., 1995. Charybdotoxin and its effects on potassium channels. Am. J. Physiol. 269, C1–C10.

Garland, C.J., Plane, F., Kemp, B.K., Cocks, T.M., 1995. Endothelium-dependent hyperpolarization: a role in the control of vascular tone. Trends Pharmacol. Sci. 16, 23–30.

Hecker, M., Bara, A.T., Bauersachs, J., Busse, R., 1994. Characterization of endothelium-derived hyperpolarizing factor as a cytochrome P-450-derived arachidonic acid metabolite in mammals. J. Physiol. 481, 407–414.

Holland, M., Langton, P.D., Standen, N.B., Boyle, J.P., 1996. Effects of BK_{Ca} channel activator, NS1619, on rat cerebral artery smooth muscle. Br. J. Pharmacol. 117, 19–29.

Honda, H., Unemoto, T., Kogo, H., 1999. Different mechanisms for testosterone-induced relaxation of aorta between normotensive and spontaneously hypertensive rats. Hypertension 34, 1232–1236.

Honda, H., Moroe, H., Kogo, H., 2001. Short term hypercholesterolemia alters N^G-nitro-L-arginine- and indomethacin-resistant endothelium-dependent relaxation by acetylcholine in rabbit renal artery. Jpn. J. Pharmacol. 85, 203–206.

Ikeda, H., Taketomi, S., Sugiyama, Y., Shimura, Y., Sohda, T., Meguro, K., Fujita, T., 1990. Effects of pioglitazone on glucose and lipid metabolism in normal and insulin resistant animals. Arzneimittel-Forschung 40, 156–162.

Inoue, I., Goto, S., Matsunaga, T., Nakajima, T., Awata, T., Hokari, S., Komoda, T., Katayama, S., 2001. The ligands/activators for peroxisome proliferator-activated receptor alpha (PPARalpha) and PPARgamma increase Cu^{2+}, Zn^{2+}-superoxide dismutase and decrease p22phox message expressions in primary endothelial cells. Metabolism 50, 3–11.

Ishii, K., Chang, B., Kerwin, J.F., Hung, Z.J., Murad, F., 1990. N^G-nitro-L-arginine: a potent inhibitor of endothelium-derived relaxing factor formation. Eur. J. Pharmacol. 176, 219–223.

Jayakody, L., Senaratne, M., Thomson, A., Kappagoda, T., 1987. Endothelium-dependent relaxation in experimental atherosclerosis in the rabbit. Circ. Res. 60, 251–264.

Kotchen, T.A., Zhang, H.Y., Reddy, S., Hoffmann, R.G., 1996. Effect of pioglitazone on vascular reactivity in vivo and in vitro. Am. J. Physiol. 20, R660–R666.

Kristof, A.S., Noorhosseini, H., Hussain, S.N., 1997. Attenuation of endothelium-dependent hyperpolarizing factor by bacterial lipopolysaccharides. Eur. J. Pharmacol. 328, 69–73.

Kuhberger, E., Groschner, K., Kukovetz, W.R., Brunner, F., 1994. The role of myoendothelial cell contact in non-nitric oxide-, non-prostanoid-mediated endothelium-dependent relaxation of porcine coronary artery. Br. J. Pharmacol. 113, 1289–1294.

Law, R.E., Meehan, W.P., Xi, X.P., Graf, K., Wuthrich, D.A., Coats, W., Faxon, D., Hsueh, W.A., 1996. Troglitazone inhibits vascular smooth muscle cell growth and intimal hyperplasia. J. Clin. Invest. 98, 1890–1897.

Ludmer, P.L., Selwyn, A.P., Shook, T.L., Wayne, R.R., Mudge, G.H., Alexander, R.W., Ganz, P., 1986. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. N. Engl. J. Med. 315, 1046–1051.

Maeshiba, Y., Kiyota, Y., Yamashita, K., Yoshimura, Y., Motohashi, M., Tanayama, S., 1997. Disposition of the new anti diabetic agent pioglitazone in rats, dogs and monkeys. Arzneimittel-Forschung 47, 29–35.

Mommbouli, J.V., Vanhotte, P.M., 1997. Endothelium-derived hyperpolarizing factor (s): updating the unknown. Trends Pharmacol. Sci. 18, 252–256.

Moncada, A., Higgs, S., 1993. The L-arginine–nitric oxide pathway. N. Engl. J. Med. 329, 2002–2012.

Moncada, S., Herman, A.G., Higgs, E.A., Vane, J.R., 1977. Differential formation of prostacyclin (PGX or PGI_2) by layers of the arterial wall. An explanation for the anti-thrombotic properties of vascular endothelium. Thromb. Res. 11, 323–344.

Mugge, A., Brandes, R.P., Boger, R.H., Dwenger, A., Bode-Boger, S., Kienke, S., Frolich, J.C., Lichtlen, P.R., 1994. Vascular release of superoxide radicals is enhanced in hypercholesterolemic rabbits. J. Cardiovasc. Pharmacol. 24, 994–998.

Najibi, S., Cohen, R.A., 1995. Enhanced role of potassium channels in relaxations of hypercholesterolemic rabbit carotid artery to NO. Am. J. Physiol. 269, H805–H811.

Nakashima, M., Mombouli, J.V., Taylor, A.A., Vanhotte, P.M., 1993. Endothelium-dependent hyperpolarization caused by bradykinin in human coronary arteries. J. Clin. Invest. 92, 2867–2871.

Ohara, Y., Peterson, T.E., Harrison, D.G., 1993. Hypercholesterolemia increases endothelial superoxide anion production. J. Clin. Invest. 91, 2546–2551.

Randall, M.D., Alexander, S.P., Bennett, T., Boyd, E.A., Fry, J.R., Gardiner, S.M., Kemp, P.A., McCulloch, A.I., Kendall, D.A., 1996. An endogenous cannabinoid as an endothelium-derived vasorelaxant. Biochem. Biophys. Res. Commun. 229, 114–120.

Standen, N.B., Quayle, J.M., Davies, N.W., Brayden, J.E., Huang, Y., Nelson, M.T., 1989. Hyperpolarizing vasodilators activate ATP-sensitive K^+ channels in arterial smooth muscle. Science 245, 177–180.

Taylor, S.G., Weston, A.H., 1988. Endothelium-derived hyperpolarizing factor: a new endogenous inhibitor from the vascular endothelium. Trends Pharmacol. Sci. 9, 272–274.

Unemoto, T., Honda, H., Kogo, H., 2003. Differnces in the mechanisms for relaxation of aorta induced by 17beta-estradiol or progesterone between normotensive and hypertensive rats. Eur. J. Pharmacol. 472, 119–126.

Verbeuren, T.J., Jordaens, F.H., Van Hove, C.E., Van Hoydonck, A.E., Herman, A.G., 1990. Release and vascular activity of endothelium-derived relaxing factor in atherosclerotic rabbit aorta. Eur. J. Pharmacol. 191, 173–184.