Short Term Hypercholesterolemia Alters $N^G$-Nitro-L-arginine- and Indomethacin-Resistant Endothelium-Dependent Relaxation by Acetylcholine in Rabbit Renal Artery

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ABSTRACT—The tension of isolated rings was measured isometrically to compare the $N^G$-nitro-L-arginine- and indomethacin-resistant relaxation by acetylcholine (ACh) in the renal artery from normal rabbits and short term hypercholesterolemia rabbits (0.5% cholesterol chow for 5 weeks). ACh-induced relaxation in the renal artery precontracted with phenylephrine was not influenced by cholesterol-enriched chow. However, in comparison with artery from normal rabbits, the $N^G$-nitro-L-arginine- and indomethacin-resistant endothelium-dependent relaxation by ACh was significantly enhanced by the chow. The resistant part of ACh-induced relaxation was significantly inhibited when the artery was treated with tetraethylammonium or SKF 525a. Results suggest that short term hypercholesterolemia modulates endothelium-derived hyperpolarizing factor-mediated relaxation in rabbit renal artery.

Keywords: Hypercholesterolemia, Renal artery, Endothelium-derived hyperpolarizing factor

It is well known that the endothelium plays an important role in the management of vascular smooth muscle tone. There are three different relaxing factors in the endothelium: prostacycline, a cyclooxygenase metabolite (1); nitric oxide (NO), an endothelial NO synthase product (2); and endothelium-derived hyperpolarizing factor (EDHF), which is thought to be a cytochrome P-450 monooxygenase-arachidonic acids metabolite (3) and suggested to belong to the group of epoxyeicosatrienoic acids (4, 5). It has been postulated that EDHF exists in the vasorelaxation induced by acetylcholine (ACh) and that it is resistant to $N^G$-nitro-L-arginine (L-NOARG), an endothelial NO synthase inhibitor, and indomethacin, a cyclooxygenase inhibitor (6, 7). The resistant part has been reported to be associated with the hyperpolarization of smooth muscle cells through the inhibition of calcium activated potassium channels (6 – 8). Chronic hypercholesterolemia is associated with an impairment of endothelium-dependent relaxations such as NO- and EDHF-mediated relaxations (9 – 11). The aim of the present study was, therefore, to determine if there is an alteration in L-NOARG and indomethacin-resistant endothelium-dependent relaxation in short term hypercholesterolemic rabbit renal artery.

Ten-week-old male Jla:JW rabbits supplied by Japan Laboratory Animal, Inc. (Tokyo) were used. Rabbits were fed either normal rabbit chow (control group) or chow enriched with cholesterol (cholesterol group, 0.5% for 5 weeks; Funabashi Nojou, Chiba). 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: 118.0 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl$_2$, 1.2 mM MgSO$_4$, 25.0 mM NaHCO$_3$ and 11 mM glucose, at 37°C gassed with 95% O$_2$ and 5% CO$_2$. The tissue was 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; Nihon Kohden, Tokyo) (12). The resting tension was 1.5 g and each preparation was equilibrated in the 10 ml bathing solution for 60 – 90 min before the experiment. Isometric tension changes were recorded on a polygraph (RECTI-HORIZ-8K; San-ei, Tokyo). After equilibration, the rings were exposed to KCl (50 mM). When the contractile responses plateaued, the rings were rinsed with modified Krebs-Henseleit solution and allowed to equilibrate for 20 minutes.
additional 60 min before the application of phenylephrine (PE, $3 \times 10^{-7}$ M). For relaxation studies, the submaximal tone was induced with PE ($3 \times 10^{-7}$ M) and then ACh was added in a cumulative fashion. L-NOARG, indomethacin, tetraethylammonium (TEA) or SKF 525a ($N,N$-diethylaminoethyl-2,2-diphenylvalerate hydrochloride) was added to the solution 10 min before treatment with PE. PE, ACh, L-NOARG, TEA or SKF 525a (Sigma, St. Louis, MO, USA) was dissolved in distilled water. Indomethacin was dissolved in 4% (w/v) NaHCO$_3$. The other chemicals were of analytical grade and obtained from Wako Pure Chem. Co., Ltd. (Osaka). The response to ACh was expressed as the percent relaxation of the PE-induced active tone. Blood samples were collected in tubes from each rabbit before excision of the renal artery. After centrifugation at 1200 $\times$ g for 15 min, the total serum cholesterol and triglyceride concentrations were measured by the standard enzymatic method. Values were expressed or plotted as the mean $\pm$ S.E.M. Statistical analysis was performed with Student’s t-test or the multiple Dunnett test, and the differences were considered significant for $P<0.05$.

Serum total cholesterol levels were significantly elevated in rabbits fed a high cholesterol diet for 5 weeks, but serum triglyceride levels did not differ between control and cholesterol-fed rabbits (Table 1). The tension induced by KCl (50 mM) was 5.14 $\pm$ 3.62 and 5.93 $\pm$ 4.62 g in control and cholesterol-fed rabbits, respectively. The tension induced by PE ($3 \times 10^{-7}$ M) was 2.40 $\pm$ 3.11 and 2.84 $\pm$ 3.12 g in control and cholesterol-fed rabbits, respectively. No difference in both contractions existed between control and cholesterol-fed rabbits. Addition of ACh ($10^{-9} - 3 \times 10^{-8}$ M) produced a dose-dependent relaxation in the renal artery rings from both control and cholesterol-fed rabbits (Fig. 1A). There was no difference in ACh-induced relaxation of the renal artery between control and cholesterol-fed rabbits. No difference in sodium nitroprusside-induced vasorelaxation also existed between control and cholesterol-fed rabbits (data not shown). In the presence of L-NOARG ($10^{-4}$ M) and indomethacin ($10^{-5}$ M), the vasorelaxation induced by ACh was significantly suppressed in both control and cholesterol-fed rabbits (Fig. 1B). L-NOARG- and indomethacin-resistant relaxation induced by ACh was significantly greater in the rings from cholesterol-fed rabbits than in those from control rabbits, and the resistant part of ACh-induced relaxation was significantly impaired after addition of either TEA ($10^{-3}$ M) or SKF 525a ($10^{-4}$ M) in both control (Fig. 2A) and cholesterol-fed rabbits (Fig. 2B).

The present results indicated that short term hypercho-

![Figure 1](image1.png)

**Fig. 1.** Acetylcholine-induced relaxation of renal artery rings precontracted with phenylephrine in control and cholesterol-fed rabbits in the absence (A) or the presence (B) of $N,N$-nitro-L-arginine (L-NOARG) and indomethacin. L-NOARG ($10^{-4}$ M) and indomethacin ($10^{-5}$ M) were administered 10 min before phenylephrine ($3 \times 10^{-7}$ M). Each value is the mean $\pm$ S.E.M. from 5 – 15 experiments. Control (○), Hypercholesterolemia (●). **$P<0.01$, from control.

<table>
<thead>
<tr>
<th>Rabbits</th>
<th>Body weight (kg)</th>
<th>Total cholesterol (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.47 ± 1.37</td>
<td>52.8 ± 9.0</td>
<td>40.3 ± 6.7</td>
</tr>
<tr>
<td>Cholesterol-fed</td>
<td>3.37 ± 0.08</td>
<td>1117.5 ± 231.8**</td>
<td>42.5 ± 9.5</td>
</tr>
</tbody>
</table>

Table 1. Serum cholesterol and triglyceride levels

Each value is the mean $\pm$ S.E.M. from 6 rabbits. **$P<0.01$, from the control.
Short Communication

Lesterolemia (0.5% cholesterol enriched chow for 5 weeks) had no influence on the relaxation induced by ACh in rabbit renal arteries, but it significantly enhanced the \( \text{L-NOARG} \) and indomethacin-resistant relaxation to ACh. It is of interest that short term hypercholesterolemia enhances the \( \text{L-NOARG} \) and indomethacin-resistant relaxation to ACh, although ACh-induced vasorelaxation was not affected by it, and no atherosclerotic lesions were observed in the renal artery isolated from rabbits that consumed a high-cholesterol chow for 5 weeks (data not shown). The resistant part of ACh-induced relaxation is suggested to be endothelium-dependent, since it was abolished by the denudation of the endothelium (data not shown). Brandes et al. reported (13) that when ACh-induced relaxation in the renal artery was inhibited by long term hypercholesterolemia rabbits (1% cholesterol chow for 4 weeks plus 0.5% cholesterol chow for 12 weeks), \( \text{L-NOARG} \) and indomethacin-resistant endothelium-dependent vasorelaxation to ACh was enhanced. The present study is the first paper showing that \( \text{L-NOARG} \) and indomethacin-resistant endothelium dependent vasorelaxation to ACh in the renal artery was enhanced by the short term hypercholesterolemia rabbits, even though ACh-induced relaxation is not affected. The enhancement in the resistant part of ACh-induced vasorelaxation may not be due to the alteration in the contracting and relaxing function of arterial smooth muscle, because potassium- or PE-induced contraction and sodium nitroprusside-induced relaxation in the renal artery are not affected by the short term hypercholesterolemia rabbits.

\( \text{L-NOARG} \) and indomethacin-resistant endothelium-dependent relaxation to ACh, which involves calcium-activated potassium channels, is assumed to be mediated by EDHF (13). In our present study, the resistant part of ACh-induced relaxation was significantly inhibited when the artery was treated with TEA, an inhibitor of calcium-activated potassium channels (14), or SKF 525a, an inhibitor of cytochrome P-450 monooxygenase (13), suggesting that the resistant part of ACh-induced relaxation may involve the opening of calcium-activated potassium channels by cytochrome P-450 monooxygenase-arachidonic acids metabolites. The enhancement of EDHF component in rabbit renal artery by short term hypercholesterolemia may be due to the attenuation of NO activity, since it is suggested that a decreased activity of NO may increase vasorelaxation mediated by EDHF (15).

In conclusion, our present results suggest that short term hypercholesterolemia modulates EDHF-mediated relaxation in rabbit renal artery, although ACh-induced vasorelaxation was not affected by it at that time. This may reflect that EDHF, instead of NO, manages the vascular tone of rabbit renal artery in short term hypercholesterolemia.

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**Fig. 2.** Effects of tetraethylammonium (TEA) and SKF 525a on the relaxation induced by acetylcholine in control (A) and cholesterol-fed rabbits (B). \( \text{L-NOARG} \) \( (10^{-5} \text{M}) \) and indomethacin \( (10^{-5} \text{M}) \) with TEA \( (10^{-3} \text{M}) \) or SKF 525a \( (10^{-5} \text{M}) \) were administered 10 min before phenylephrine \( (3 \times 10^{-7} \text{M}) \). Each value is the mean ± S.E.M. from 8 – 15 experiments. \( \text{L-NOARG} \) + indomethacin (○), \( \text{L-NOARG} \) + indomethacin + TEA (△), \( \text{L-NOARG} \) + indomethacin + SKF 525a (□). \(*P<0.05, \quad **P<0.01 \) from \( \text{L-NOARG} \) + indomethacin.
REFERENCES


