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Effects of Doxazosin on Functional Alterations of Isolated Coronary Arteries from Cholesterol-fed Rabbits

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Abstract

Anti-hypertensive treatment is much less successful at reducing coronary artery disease than at reducing mortality from stroke and congestive heart failure. The effects of the α-adrenergic antagonist doxazosin on progression of atheromatous lesions and functional responses of isolated coronary arteries from cholesterol-fed rabbits have been investigated.

Normotensive rabbits were fed either a standard chow (control, n = 8) or a 1% cholesterol-rich diet (n = 16) for 20 weeks. After 3 weeks the cholesterol-fed animals were assigned randomly to two groups either given placebo capsules (n = 8) or treated with doxazosin (5 mg kg^{-1} day^{-1}; n = 8). Doxazosin reduced the mean arterial blood pressure by 10% that of the control and placebo-treated cholesterol-fed rabbits, but did not affect the plasma cholesterol, triacylglycerol and phospholipid levels, which were, after 20 weeks, severalfold increased in the cholesterol-fed rabbits compared with controls. Histological examination showed atheromatous lesions in proximal (but not distal) coronary arteries from both groups of cholesterol-fed rabbits. Doxazosin either had no effect on reduced contractions to 125 mmol L^{-1} potassium saline solution or increased contractions to 5-hydroxytryptamine in proximal isolated coronary arteries from the cholesterol-fed rabbits. It did, however, abolish the hyper-responsiveness of the large atheromatous coronary arteries to noradrenaline. In both vehicle- and doxazosin-treated cholesterol-fed rabbits the maximum relaxation and sensitivity to acetylcholine were significantly reduced in proximal segments compared with the control group, whereas responses to acetylcholine in distal coronary segments were not significantly different. The relaxation to sodium nitroprusside, adenosine diphosphate and isoprenaline in proximal and distal coronary arteries were similar in the three experimental groups.

These results indicate that treatment of normotensive cholesterol-fed rabbits with doxazosin prevents the hyper-responsiveness to noradrenaline of proximal coronary arteries, although it does not prevent the progression of other functional alterations observed in the coronary circulation.

Anti-hypertensive treatment, which is successful in reducing mortality from stroke and congestive heart failure, is much less effective in reducing coronary artery disease. The coronary morbidity in hypertension may not be a direct consequence of blood pressure elevation, but rather of atherogenic non-pressure-related risk factors such as overweight, higher insulin and increased cholesterol and triglyceride levels (Julius 1993).

Impairment of endothelium-dependent relaxation, which is an early marker of atherosclerosis (Jayakody et al 1988; Ross 1993), and progression of atherosclerotic lesions can be suppressed by treatment with cholesterol-reducing drugs both in cholesterol-fed rabbits (Osborne et al 1989) and in hypercholesterolaemic patients (Leung et al 1993; Egashira et al 1994). Oxidatively modified lipoproteins seem to be a key component in endothelial dysfunction (Ross 1993), and several antioxidants have recently been shown to preserve endothelium-dependent relaxation in atherosclerotic rabbit aortae (Keaney et al 1993; Simon et al 1993; Stewart-Lee et al 1994). Atherogenesis in cholesterol-fed rabbits can also be suppressed without reducing serum cholesterol by treatment with drugs interacting with the accumulation of calcium in the arteries, although lack of anti-atherosclerotic effect of calcium antagonists has also been reported (Waters & Lesperance 1994). Calcium antagonists have also been reported to preserve endothelium-dependent relaxation to acetylcholine in aortae from cholesterol-fed rabbits (Habib et al 1986; Kappagoda et al 1991; Riezebos et al 1994).

The centrally acting adrenergic inhibitor, reserpine, and the peripheral sympathetic blocker guanethidine, which alter vascular calcium in experimental atherosclerosis, were reported to retard atherogenesis in the cholesterol-fed rabbit (Whittington-Coleman et al 1968; Whittington-Coleman & Carrier 1970). The anti-atherosclerotic effect of these drugs might, however, also be ascribed to their anti-adrenergic action.

The adrenergic antagonist, doxazosin, used clinically to lower blood pressure, was recently found to inhibit the development of atheromatous lesions in the aortic arch of cholesterol-fed hamsters (Kowala & Nicolosi 1989; Foxall et al 1992) and suppress the accumulation of cholesterol and formation of atherosclerotic plaques in aortae of cholesterol-fed rabbits, independent of plasma lipid concentrations and blood pressure (Swindell 1988; Swindell et al 1993). The hydroxy metabolites of doxazosin, moreover, inhibit low-density lipoprotein oxidation in-vitro (Chait et al 1994), and doxazosin enhances the activity of endothelium-derived relaxing factor (EDRF) in normal rabbit aortae in-vitro (Yeates 1994). The effect of antagonists on either the progression of atherosclerosis or functional alterations in the coronary circulation following cholesterol feeding of rabbits has, however, not yet been examined. This study was, therefore, designed to investigate whether a dose of doxazosin (5 mg kg^{-1} day^{-1}), giving plasma concentrations in the therapeutic range (Conrad et al 1988), protects against

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alterations in either vessel wall structure or functional responses to contractile and relaxant agonists in the coronary circulation of normotensive cholesterol-fed rabbits.

Materials and Methods

Animals

Male New Zealand White rabbits, 24-weeks old, housed identically in single cages, were fed either standard rabbit chow (Ewos, Sädertalje, Sweden; control group, C, n = 8) or 1% cholesterol-enriched diet (n = 16) for 20 weeks. The atherogenic diet was prepared every week by dissolving 1% cholesterol by weight in diethyl ether (Rathburn, 99%, glass-distilled), soaking the standard rabbit chow with this mixture, and letting the ether evaporate in a fume cupboard as earlier described (Simonsen et al 1992). The food was restricted to 110 g but water was freely available. After a 3-week period the cholesterol-fed animals were randomly assigned to two groups: a cholesterol-fed group given placebo capsules (CH, n = 8); and a cholesterol-fed group treated with doxazosin (5 mg kg^{-1} day^{-1}; DCH, n = 8). The control group was also given placebo capsules. The gelatin capsules either containing doxazosin, or lactulose monohydrate (Merck) as substitute, were administered each day between 0900 and 1000 h by placing them at the back of the throat of the rabbit with a finger and waiting until the animal has swallowed the capsule. The housing and experimental procedures were in accordance with Danish Animal Law and Regulations.

Plasma analysis

Blood samples for determination of plasma cholesterol, triacylglycerol and phospholipid concentrations were taken just before starting the feeding, after 3, 6 and 12 weeks, and at time of killing. The concentrations were measured enzymatically (Chod-PAP, GPO-PAP High performance, Boehringer Mannheim GmbH, FRG).

Systemic haemodynamics

Heart rate was determined by use of a stethoscope and the mean arterial blood pressure was measured by introducing a catheter into the central ear artery of unanaesthetized animals 3 h after the last administration either of placebo or capsules containing doxazosin. The catheter was connected to a transducer (Medex, Novotrans MX 800, Simonsen and Weel, Denmark) and the signal was recorded on a Servogor 511 recorder.

Solutions

Unless otherwise stated, the vessels were dissected, mounted and held relaxed in a physiological salt solution (PSS) of composition (mM): NaCl 119, $NaHCO_3$ 14·9, KCl 4·7, KH_2PO_4 1·18, $MgSO_4$ 1·17, $CaCl_2$ 2·5, ethylenediaminetetraacetic acid (EDTA) 0·026 and glucose 5·5. Potassium PSS (K^+PSS) was the same as PSS, but with an equimolar exchange of NaCl for KCl. NA-PSS was PSS to which 10 μm noradrenaline was added and NAK was K^+PSS containing 10 μm noradrenaline. All solutions were kept at 37°C, pH = 7·4 and bubbled with 5% CO_2 and 95% O_2. Drugs used were noradrenaline hydrochloride (Sigma), propranolol (Frekven, Ferrosan, Copenhagen, Denmark), acetylcholine (Fluka AG, Buchs, Switzerland), sodium nitroprusside dihydrate (Merck), 5-hydroxytryptamine creatinine sulphate complex (5-HT, Sigma), adenosine diphosphate (Sigma), isoprenaline (Sigma), substance P (Sigma). Stock solutions were made either daily or thawed from a freezer, where they were kept at −20°C for a maximum of two weeks.

With the exception of 5-HT, which was dissolved in PSS, solutions were prepared in distilled water. Dilutions were made immediately before the experiment. All concentrations are expressed as the final concentrations in the tissue bath fluid.

Dissection and mounting

After 20 weeks of receiving the respective diets, two rabbits, one from each group, were killed by cervical dislocation each day. The hearts were rapidly excised and placed in ice-cold PSS to reduce cardiac metabolism and anoxia. Throughout the subsequent dissection, the hearts were bathed in cold (4°C) PSS. Segments (approx. 2 mm long) of the proximal and distal part of the left anterior descending coronary artery were dissected as previously described (Simonsen et al 1992). The vessels were subsequently mounted as ring preparations on two 40-μm wires in an isometric myograph by fixing one of the wires to a force transducer and the second to a length displacement device (Mulvany & Halpern 1977). In all cases, a double myograph was used (Mulvany & Nyborg 1980). Segments from control rabbits (C), cholesterol-fed rabbits (CH), and cholesterol-fed rabbits treated with doxazosin (DCH) were assigned randomly and mounted at the near or far force transducer, which enabled two vessel segments to be examined simultaneously and exposed to the same solutions.

Normalization and standardization of the response

As previously described (Mulvany & Halpern 1977), the vessels were set to the internal circumference L_1, given by $L_1 = 0{\cdot}9 \times L_{100}$, where internal circumference L_{100} corresponds to a transmural pressure of 100 mmHg for a relaxed vessel in-situ. At L_1 the force production in rabbit coronary arteries is close to maximum (Simonsen et al 1992). The effective lumen diameter of the vessels is expressed as $l_1 = L_1/\pi$. The experiments with the coronary vessels were initiated by activation three times with K^+PSS at 5 min intervals to check their mechanical condition. The tissue maximum force development of these vessels was obtained by stimulating with K^+PSS containing 10^{-5} M prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). From the response measured at the end of each stimulating period the active wall tension, δT, which is the increase in measured force, δF, divided by twice the segment length, was calculated (Mulvany & Halpern 1977).

Functional studies

The proximal and distal coronary arteries were exposed to the same protocol. For determination of relaxation responses, the normal PSS was replaced by 30 mmol L^{-1} K^+PSS. The bath volume was 14 mL and when a plateau was reached, cumulative relaxation curves were constructed by adding aliquots of the relaxing agonist: a concentration–response curve to acetylcholine (10^{-9}–10^{-4} mol L^{-1}); a concentration–response curve to sodium nitroprusside (10^{-8}–3×10^{-5} mol L^{-1}); a concentration-response curve to isoprenaline; a concentration–response curve to adenosine diphosphate (ADP). In addition, cumulative concentration-response curves were constructed: 5-HT (10^{-9} –3×10^{-5} mol L^{-1}); noradrenaline (10^{-8}–3×10^{-5} mol L^{-1}) in presence of propranolol 10^{-5} mol L^{-1} to block the β-receptors. On completion of the mechanical experiments with

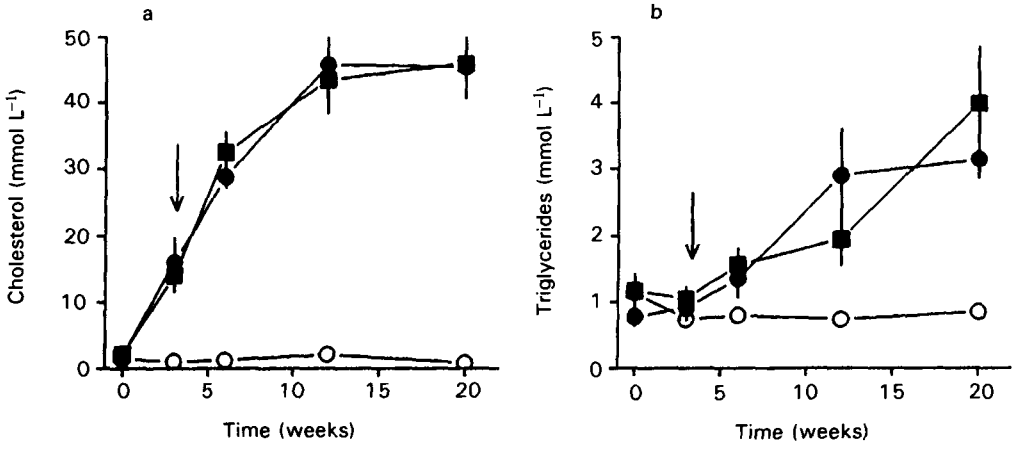

FIG. 1. Plasma lipid concentrations (a, cholesterol; b, triglycerides) in control rabbits (○), cholesterol-fed rabbits treated with placebo (●), and the cholesterol-fed doxazosin-treated group (■). The arrows at three weeks indicate the start of doxazosin or placebo treatment. Each point is mean ± s.e.m. of 6–8 rabbits.

the coronary arteries in the myograph, the solution was changed to calcium-free PSS for 10 min to obtain complete relaxation.

Histological examination

The vessels were fixed for histology, while still mounted on the myograph at the internal circumference L_1, using 5% glutaraldehyde in Sørensen's Buffer adjusted to pH 7·4. The vessels were unmounted and post-fixed in glutaraldehyde and pre-embedded in agar to maintain orientation, dehydrated in ethanol and embedded in historesin, before 5-μm transverse sections were performed. The sections were mounted on slides and stained with Giemsa for light microscopy.

Data and statistical analysis

The mechanical responses of the vessels were measured as force and expressed as active wall tension, δT, which is the increase in measured force, δF, divided by twice the segment length (Mulvany & Halpern 1977). For each concentration–response curve, the concentration required to give half maximum response (EC50) was determined by computerized iteration (GraphPad Software version 4.0, San Diego, USA), fitting the responses and logarithmic concentrations to the Hill equation. EC50 values are expressed as the negative log molar concentration, $_pD_2 = -\log$ (EC50). The responses to the relaxing agonists were normalized to the initial tone in the vessel induced with 30 mmol L^{-1} K^+PSS and the concentration to give half maximum response was calculated and expressed as pIC50 (i.e. the negative log molar concentration). The results are expressed as means ± standard error of the mean (s.e.m.; number of animals). The areas under the concentration–response curves, or indicated parts of them, of each individual experiment (i.e. area in arbitrary values) were used for comparison (Simonsen et al 1992). Differences between group means were tested using the Student-Newman-Keuls test for subsequent ranking of groups (Sokal & Rohlf 1969). Probability levels under 5% were considered significant.

Results

General parameters

After a 3-week feeding period, the plasma levels of cholesterol and triacylglycerol were equal in the cholesterol-fed rabbits allocated for treatment with either doxazosin or placebo capsules; the plasma cholesterol concentration was 14·1 ± 2·6 (n = 8) and 15·0 ± 3·7 mmol L^{-1} (n = 8) for the doxazosin-treated (DCH) and placebo-treated rabbits (CH), respectively, whereas the triacylglycerol levels were 1·05 ± 0·19 (n = 8) and 0·91 ± 0·04 mmol L^{-1} (n = 8), respectively (Fig. 1). The plasma levels of cholesterol and triacylglycerol in the control rabbits were unaltered throughout the study. After 5-weeks study, one rabbit from the placebo-treated cholesterol-fed group died from vestibular dysfunction. After 20 weeks plasma cholesterol, triacylglycerol and phospholipid levels were increased several times in the cholesterol-fed rabbits compared with control levels (Table 1). There was no difference in plasma-lipid levels in cholesterol-fed rabbits treated with either placebo or doxazosin. The mean arterial blood pressure was non-significantly reduced by an average of 10·5% compared with the control or placebo cholesterol-fed rabbits, whereas body weight, heart weight and heart rate were unaltered by cholesterol feeding or doxazosin treatment (Table 1). The livers of the hypercholesterolaemic rabbits were yellow and fatty, and their weights were significantly increased compared with those of control rabbits (Table 1). In all cholesterol-fed rabbits round white precipitates were, furthermore, observed in the iris of the eyes, whereas the macroscopic appearance of the eyes of the control rabbits was unaltered throughout the study.

Functional studies of coronary arteries

The effective internal lumen diameters l_1 of the proximal epicardial part of the left anterior descending coronary artery in the control (C), cholesterol-fed (CH) and cholesterol plus doxazosin (DCH) rabbits were 1431 ± 65 (n = 8), 1408 ± 94 (n = 7) and 1423 ± 64 μm (n = 8), respectively. In the distal intramyocardial coronary segments the internal diameters were also similar in the three experimental groups; 348 ± 55 (n = 7, C), 418 ± 37 (n = 6, CH), and 411 ± 47 μm (n = 7, DCH). The resting tension applied to the proximal coronary and distal coronary arterial segments for functional studies and the myogenic tension, measured as the difference between the baseline just after normalization compared with the baseline obtained after exchange of PSS for Ca^{2+}-free PSS, were not different in

Table 1. General parameters in control, 1% cholesterol-fed, and doxazosin-treated cholesterol-fed New Zealand White rabbits after a 20-week feeding period.

	Control	Cholesterol-fed	Doxazosin-treated
Plasma cholesterol (mmol L^{-1})	0·79 ± 0·06 (8)	45·4 ± 4·1 (7)*	45·9 ± 5·3 (8)
Plasma triacylglycerol (mmol L^{-1})	0·85 ± 0·07 (8)	3·14 ± 0·09 (7)*	3·99 ± 0·87 (8)*
Plasma phospholipids (mmol L^{-1})	0·12 ± 0·03 (8)	4·61 ± 1·0 (7)*	4·94 ± 0·96 (8)*
Body weight (kg)	3·7 ± 0·1 (8)	3·6 ± 0·1 (7)	3·5 ± 0·01 (8)
Heart weight (g)	12·2 ± 0·9 (8)	13·2 ± 0·8 (7)	12·3 ± 0·6 (8)
Liver weight (g)	130·2 ± 3·3 (8)	171·4 ± 6·2 (7)*	175·2 ± 18·1 (8)*
Heart rate (beats min^{-1})	134 ± 5 (8)	135 ± 4 (7)	144 ± 7 (8)
Mean arterial pressure (mmHg)	106 ± 5 (6)	106 ± 5 (5)	95 ± 11 (6)

Values are mean ± s.e.m.; (n) is the number of animals examined for each value. Statistical differences evaluated with one-way analysis of variance followed by the Student-Newman-Keuls test: *$P < 0·05$ vs control animals; **$P < 0·05$ vs cholesterol-fed animals.

proximal and distal coronary arterial segments from the three groups (data not shown). In contrast, the responses to 125 mmol L^{-1} K^+PSS alone and with 10^{-5} mol L^{-1} $PGF_{2\alpha}$ added (giving the maximum active tension) were both diminished in proximal segments from both treated and placebo-fed hypercholesterolaemic rabbits. The magnitude of the K^+PSS-induced contraction was, therefore, 6·7 ± 1·0 N m^{-1} (n = 8) in the control group, 3·9 ± 0·8 N m^{-1} (n = 7, $P < 0·05$) in the cholesterol-treated group, and 3·8 ± 0·7 N m^{-1} ($P < 0·05$, n = 8) in the cholesterol- plus doxazosin-treated group. In distal segments the contractions induced by 125 mmol L^{-1} K^+PSS were similar in the three experimental groups: 3·4 ± 0·4 (n = 7, C), 3·5 ± 0·4 (n = 6, CH), and 3·9 ± 0·6 N m^{-1} (n = 6, DCH).

Contractile responses to noradrenaline, 5-HT and U46619

Contractile responses to noradrenaline were significantly increased in proximal segments of cholesterol-fed rabbits compared with the control group (Fig. 2a), and treatment of the cholesterol-fed rabbits with doxazosin prevented this hyper-responsiveness (Fig. 2a). The maximum contractions to noradrenaline in distal coronary arteries from the three experimental groups were not significantly different from baseline tension: 0·01 ± 0·01 (n = 7, C), 0·02 ± 0·01 (n = 6, CH), and 0·03 ± 0·01 N m^{-1} (n = 7, DCH). 5-HT (10^{-9}–10^{-5} mol L^{-1}) induced significantly larger contractions in proximal segments from cholesterol-fed rabbits compared with those in control rabbits (Fig. 2b), and treatment with doxazosin did not influence the increased response to 5-HT in cholesterol-fed rabbits (Fig. 2b). 5-HT induced only weak contractions in distal coronary segments from both the control and cholesterol-fed groups with a maximum of: 0·04 ± 0·01 (n = 7, C), 0·05 ± 0·03 (n = 6, CH), and 0·02 ± 0·01 N m^{-1} (n = 7, DCH). The maximum contractions to U46619 in distal coronary segments were small and not influenced by cholesterol feeding or doxazosin treatment: 0·4 ± 0·3 (n = 7, C), 1·0 ± 0·6 (n = 6, CH), and 0·1 ± 0·05 N m^{-1} (n = 7, DCH).

Relaxant responses to acetylcholine, ADP, nitroprusside and isoprenaline

Treatment with 30 mmol L^{-1} K^+PSS contracted the coronary vessels to a steady state of force. With this preconstriction, acetylcholine (10^{-8}–10^{-4} mol L^{-1}) caused concentration-dependent relaxation with both higher sensitivity and a larger maximum response in proximal coronary segments from control rabbits compared with those from the cholesterol-fed groups (Table 2). Also the areas under the curves for acetylcholine in proximal segments from the cholesterol-fed rabbits were significantly different from those for control segments (Fig. 3a). Acetylcholine (10^{-8}–10^{-4} mol L^{-1}) caused less relaxation in some distal coronary arteries from placebo-treated cholesterol-fed rabbits, but the areas under the acetylcholine curve, the pIC50, and the maximum relaxation of distal coronary arteries were not significantly different in the three experimental groups (Fig. 3b, Table 2).

ADP (10^{-8}–3×10^{-5} mol L^{-1}) evoked weak relaxation of proximal segments from both control and cholesterol-fed rabbits and relaxed the distal segments to a greater extent. There were no significant differences in the responses of distal segments to ADP from the three experimental groups of animals (Table 2). Sodium nitroprusside (SNP; 10^{-8}–10^{-4} mol L^{-1}) caused similar concentration-dependent relaxation in both proximal and distal segments (Table 2). Proximal and distal coronary segments of cholesterol-fed rabbits relaxed in the same way as segments from control animals and the treatment with doxazosin did not influence the responses to SNP. The β-adrenergic receptor agonist isoprenaline induced a concentration-dependent (10^{-9}–10^{-5} mol L^{-1}) relaxation of potassium-contracted proximal and distal coronary segments. pIC50 and maximum relaxation to isoprenaline of segments from the three experimental groups of rabbits were not different (Table 2).

Histological studies of coronary segments

In all sections of proximal coronary segments isolated from hearts of cholesterol-fed vehicle-treated (n = 4) or cholesterol-fed doxazosin-treated (n = 5) rabbits, atheromatous lesions with abnormal cell accumulations of foam cells, lymphocytes and plasma cells were observed in the developed neointima. The endothelial cell lining appeared intact except where the suspending wires had damaged the endothelium (5–10% of the luminal area), whereas the internal elastic membrane had interruptions in two of the segments examined. The nature and extension of the lesions observed in the proximal coronary segments from the two groups of cholesterol-fed rabbits were

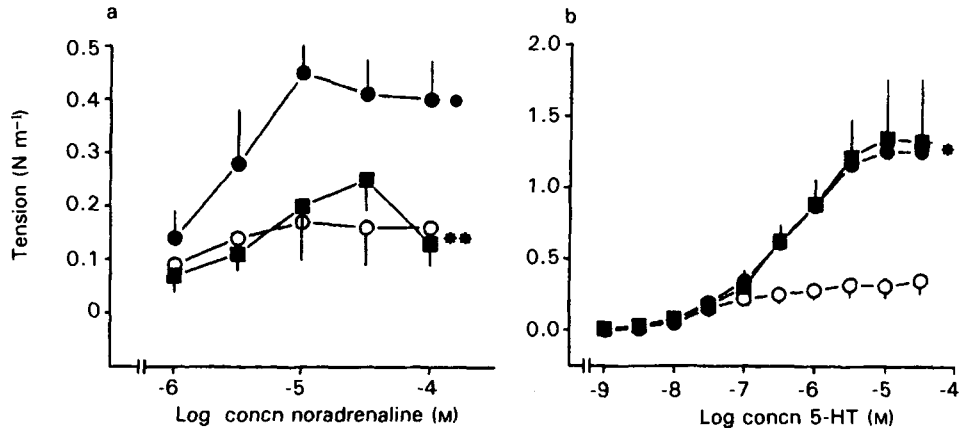

FIG. 2. Cumulative concentration-response curves for (a) noradrenaline and (b) 5-HT in proximal coronary arteries from control (○), cholesterol-fed vehicle-treated (●), and cholesterol-fed doxazosin-treated (■) rabbits. Each point is the mean ± s.e.m. of 7–8 coronary segments. $*P < 0.05$ compared to control group; $**P < 0.05$ vs cholesterol-fed animals.

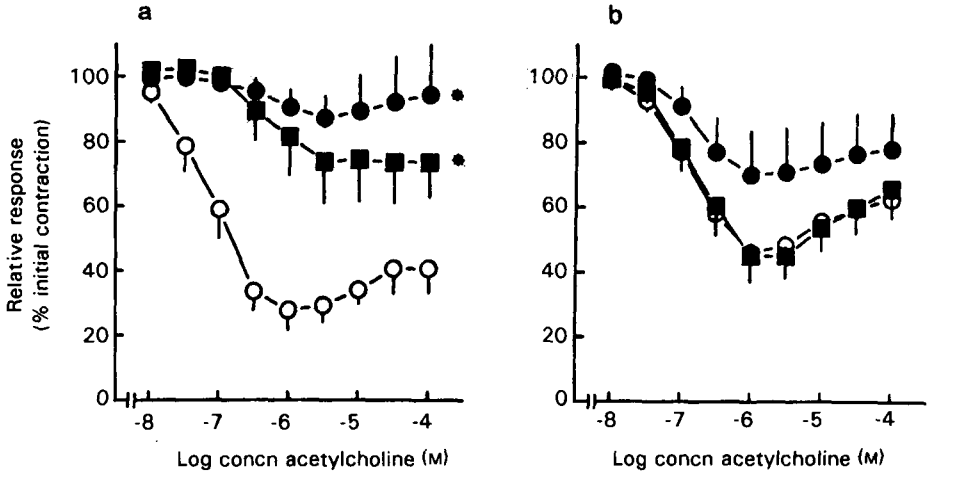

FIG. 3. Plot of endothelium-dependent relaxation to acetylcholine of potassium-contracted coronary segments from control (○), cholesterol-fed (●) and doxazosin-treated cholesterol-fed (■) rabbits. (a) Areas under the curves for acetylcholine (10^{-9}–10^{-4} mol L^{-1}) of proximal coronary artery segments from cholesterol-fed and doxazosin-treated cholesterol-fed were significantly different from the C group ($*P < 0.05$ vs control, Student-Newman-Keuls test), but there was no significant difference between the relaxation to acetylcholine of proximal coronary segments from cholesterol-fed and doxazosin-treated cholesterol-fed. (b) In distal coronary segments, the response to acetylcholine was apparently changed only in the segments from the placebo-treated cholesterol-fed rabbits, but the areas under the curves were not significantly different ($P > 0.15$ one-way analysis of variance). Results are expressed as percentage of the initial contractile response and are shown as mean ± s.e.m. of preparations from 6–8 rabbits.

similar. Atheromatous lesions or abnormal cells were not observed in distal coronary segments from the cholesterol-fed vehicle-treated (n = 4) or cholesterol-fed doxazosin-treated (n = 5) rabbits. Light microscopic examination of proximal and distal coronary segments from control rabbits (n = 4) did not reveal any atheromatous lesions or pathological alterations.

Discussion

Treatment of rabbits with induced hypercholesterolaemia with the α_1-adrenergic antagonist, doxazosin, used amounts consistent with the therapeutic range in patients receiving doxazosin as therapy for hypertension (Conrad et al 1988; Swindell et al 1993). Thus, whereas the heart rate remained unaltered, the systemic mean blood pressures of the normotensive hypercholesterolaemic rabbits in this study were reduced to an extent similar to that observed in normotensive non-human primates and patients (Elliott et al 1982).

In patients with mild to moderate hypertension, doxazosin was found either not to alter serum lipid levels (Torvik & Madsbu 1986; Svetkey et al 1988) or to have a lipid-lowering effect (Lehtonen et al 1986; Cubeddu et al 1988; Nash 1990). Doxazosin reduced serum lipid levels in hyper-cholesterolaemic non-human primates (Stucchi et al 1993), cholesterol-fed gerbils and hamsters (Kowala & Nicolosi 1989; Foxall et al

Table 2. Effects of the vasodilators acetylcholine, adenosine diphosphate (ADP), sodium nitroprusside, and isoprenaline on vascular tone of proximal and distal coronary arterial segments.

		Control		Cholesterol-fed		Doxazosin-treated	
		pIC_{50}	Maximum relaxation (%)	pICS0	Maximum relaxation (%)	pIC_{50}	Maximum relaxation (%)
Acetylcholine	proximal	7·04 ± 0·11 (8)	76·8 ± 4·3 (8)	5·40 ± 0·40 (7)*	19·1 ± 7·1 (7)*	5·64 ± 0·39 (8)*	37·4 ± 9·1 (8)*
	distal	6·84 ± 0·09 (7)	55·3 ± 5·1 (7)	6·14 ± 0·45 (6)	35·4 ± 11·6 (6)	6·72 ± 0·13 (7)	58·6 ± 7·3 (7)
ADP	proximal	—	13·1 ± 8·2 (8)	—	7·3 ± 3·6 (7)	—	12·1 ± 4·5 (8)
	distal	5·24 ± 0·17 (7)	47·6 ± 5·7 (7)	5·42 ± 0·33 (6)	50·3 ± 7·0 (6)	5·52 ± 0·04 (7)	47·3 ± 4·1 (7)
Sidium nitro	proximal	6·34 ± 0·15 (8)	80·9 ± 4·0 (8)	6·78 ± 0·19 (7)	82·9 ± 6·9 (7)	6·62 ± 0·08 (8)	81·3 ± 2·8 (8)
prussive	distal	6·25 ± 0·13 (7)	84·9 ± 2·8 (7)	6·00 ± 0·19 (6)	81·1 ± 4·6 (6)	6·05 ± 0·11 (7)	85·4 ± 2·3 (7)
Isoprenaline	proximal	6·86 ± 0·13 (8)	65·6 ± 2·2 (8)	6·84 ± 0·05 (7)	48·6 ± 5·1 (7)	7·02 ± 0·07 (8)	40·4 ± 5,9 (8)
	distal	6·65 ± 0·07 (7)	50·3 ± 5·0 (7)	6·66 ± 0·09 (6)	55·3 ± 3·7 (6)	6·69 ± 0·04 (7)	53·9 ± 7·1 (7)

Values are geometric mean ± s.e.m. The maximum relaxation is expressed as a percentage of the contraction induced by 30 mmol L^{-1} potassium physiological salt solution. pIC50 = − log IC50, where IC50 was the concentration needed to obtain half-maximum relaxation. For segments that did not relax, the IC50 was set to 10^{-4} M of the relaxing agonist. (n) is the number of coronary segments (one per animal). Statistical differences were evaluated with one-way analysis of variance followed by the Student-Newman-Keuls test: *P <0·05 vs control animals, **P <0·05 vs cholesterol-fed animals.

1992). The cholesterol-lowering effect of doxazosin reported in some studies in man or hypercholesterolaemic animal models was ascribed to an upregulation of low-density lipoprotein (LDL) receptor activity leading to reduced LDL-cholesterol plasma levels (Nash 1990; Stucchi et al 1993). In this study, the plasma concentrations of cholesterol, triacylglycerol and phospholipids of the cholesterol-fed rabbits were unaltered by treatment with doxazosin. This observation is in agreement with the lack of effects of doxazosin (Leren & Berg 1988; Swindell et al 1993) and also another α_1-adrenergic antagonist, prazosin (Minato et al 1993) in reducing increased plasma lipid levels of cholesterol-fed rabbits. This consistent lack of cholesterol-lowering effect of α_1-adrenergic antagonists in rabbits with induced hypercholesterolaemia might be ascribed to inter-species differences and a different lipid metabolism with major increases in β-very low-density lipoprotein plasma cholesterol, rather than the increases in LDL cholesterol observed in other species (Kovanen et al 1981). Although doxazosin did not alter serum levels of cholesterol or triacylglycerols in cholesterol-fed rabbits, the drug was, however, shown to have a favourable influence on the progression of atherosclerotic lesions (Swindell et al 1993).

In this study, atheroma-like lesions were primarily located in the main branches of the coronary arteries, whereas no morphological changes were observed in the small intramural coronary branches of cholesterol-fed rabbits. Doxazosin had no effect on the development of these atheromatous lesions in the proximal coronary arteries. In cholesterol-fed rabbits, atherogenesis develops simultaneously in both the coronary and aortic vascular beds (Ginsburg et al 1983; Östlund Lindquist et al 1988). In contrast with our study, doxazosin was found to reduce both cholesterol accumulation and extension of atheromatous plaques in the thoracic and abdominal aorta of cholesterol-fed rabbits (Swindell 1988; Swindell et al 1993).

Both in this study and in those performed by Swindell and coworkers (Swindell 1988, Swindell et al 1993), the rabbits were fed a 1% cholesterol-rich diet and treated with the same dose (5 mg kg^{-1} day^{-1}) of doxazosin. In some respects, the differences between the effect of doxazosin on the atheromatous lesions in the two studies could be compared with those published by Ginsburg et al (1983) comparing the effect of calcium antagonists on atherogenesis in aorta and coronary arteries from cholesterol-fed rabbits. The calcium antagonists lanthanum, diltiazem and flunarizine suppressed the development of atheromatous lesions in aortae, but had no effect on the lesions in the coronary circulation (Ginsburg et al 1983). Whereas several studies in animal models have, moreover, reported an anti-atherosclerotic effect of calcium antagonists in the rabbit aorta, no clear benefit has been shown for these drugs on progression of coronary artery disease in man (Vos et al 1993; Waters & Lesperance 1994). Similarly, the β-adrenergic antagonist, metoprolol, was reported to suppress atheromatous lesions in aorta, but had no effect on diet-induced atherosclerosis of the coronary arteries in hypercholesterolaemic rabbits (Östlund Lindquist et al 1988). Ginsburg et al (1983) suggested that coronary atherogenesis might be mechanistically different from peripheral vascular atherosclerosis, and morphological and immunohistochemical studies have recently shown that the atherosclerotic lesions of coronary arteries and aorta in Watanabe heritable hyper-lipidaemic rabbits are different (Shiomi et al 1994). Interestingly, doxazosin treatment suppresses peripheral atherosclerosis in cholesterol-fed rabbits (Swindell 1988; Swindell et al 1993). Our study, in contrast, indicates that doxazosin does not influence atherogenesis in the coronary circulation.

Atherosclerosis is associated with increased vasoconstrictor responses in the large coronary arteries of man (Ludmer et al 1986) and hypercholesterolaemic rabbits (Henry & Yokoyama 1980; Vrints et al 1990; Simonsen et al 1992). Consistent with this, in this study the contractile responses to noradrenaline and 5-HT were increased, whereas contractions in response to potassium-rich solution were reduced in the large coronary arteries with atheromatous lesions, but not in the distal coronary arteries. Doxazosin had no effect on the altered responses to

either 5-HT or potassium-rich solution, but it abolished the developed hyper-responsiveness to noradrenaline in large coronary arteries of the cholesterol-fed rabbits. Dietary treatment followed by cholesterol reduction in atherosclerotic monkeys abolished hyper-responsiveness to 5-HT in the hind limb (Heistad et al 1987), and treatment with indapamide, which has calcium antagonist properties, restored the reduced contractions in response to potassium-rich solutions in large femoral arteries from cholesterol-fed rabbits (Rio et al 1993). In the latter studies, however, both regression diet (Heistad et al 1987) and drug treatment (Rio et al 1993) also reduced the extent of atherosclerotic lesions in the arteries examined. In contrast with the reduced contractile responses to noradrenaline in the atherosclerotic aorta of cholesterol-fed rabbits (Verbeuren et al 1986), the contractility to noradrenaline has been observed to be increased in the coronary circulation of hypercholesterolaemic animals (Rosendorff et al 1981). Our results, therefore, demonstrate that although doxazosin might not have an anti-atherosclerotic effect in the coronary circulation of hyper-lipidaemic animals, it abolishes the hyperresponsiveness to noradrenaline in these arteries, which may be of potential therapeutic interest, because increased sympathetic activity has been proposed as a cause of vasospasm in large coronary arteries (Nabel et al 1988; Julius 1993).

In this study, the hyper-lipidaemia was associated with impaired endothelium-dependent relaxation to acetylcholine in the coronary circulation. The impairment was most pronounced in the proximal coronary arteries whereas it did not reach statistical significance in the distal coronary arteries of the cholesterol-fed rabbits. In cholesterol-fed rabbits, the impairment is most marked in the thoracic aorta whereas endothelium-dependent relaxation in the abdominal aorta and carotid artery are preserved (Verbeuren et al 1986; Simon et al 1993). Endothelium-dependent relaxation in the latter two preparations were recently shown to be mediated by both nitric oxide (NO) and an endothelium-derived hyper-polarizing factor (EDHF) (Cowan et al 1994), and EDHF was suggested to have an enhanced role in relaxation in response to acetylcholine in the carotid artery of hypercholesterolaemic rabbits (Najbi et al 1994). NO and EDHF have also been reported to be involved in the relaxation in response to acetylcholine in guinea-pig coronary arteries (Eckman et al 1994); the different effects on the large and small coronary arteries of cholesterol-fed rabbits in this study might be ascribed to different releases of NO and EDHF in proximal and distal coronary segments.

The data obtained suggest a trend both towards impaired endothelium-dependent relaxation to acetylcholine in distal coronary arteries of cholesterol-fed rabbits and for an improvement following treatment with doxazosin. The results did not, however, reach statistical significance; doxazosin did not, moreover, increase the reduced relaxation to acetylcholine in the proximal coronary arteries of the cholesterol-fed rabbits, where a significant effect on these relaxations was observed. This observation agrees with earlier studies showing that there is a correlation between reduction of maximum relaxation evoked by acetylcholine and fatty streak formation (Verbeuren et al 1986) and, because doxazosin did not suppress the morphological alterations observed in coronary arteries of cholesterol-fed rabbits, an effect on the impaired endothelium-dependent relaxation would not be expected. The endothelium-independent relaxation in response to ADP, SNP and isoprenaline of the coronary arteries from cholesterol-fed compared with control rabbits were not different in this study and, accordingly, treatment with doxazosin did not influence these functional responses.

In summary, this study emphasizes the need to evaluate the effects of anti-atherogenic treatments not only in aortae, but also in the clinically more relevant coronary circulation. Treatment of cholesterol-fed normotensive rabbits with doxazosin neither lowers plasma lipid levels nor suppresses the atherogenic progression, evaluated as decreased contractility to potassium solution, impaired endothelium-dependent relaxation to acetylcholine and increased contractions to 5-HT of the large coronary arteries. Doxazosin does, however, suppress hyper-responsiveness to noradrenaline in atheromatous coronary segments of cholesterol-fed rabbits and further studies must be undertaken to show whether this effect contributes to reduced non-pressure-related coronary morbidity risk in hypertensive patients.

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