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Differential Efficacy of Vasodilators in Hypercholesterolaemic Rabbits

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Abstract

The effects of hypercholesterolaemia on the endothelium-dependent and -independent vascular reactivity of the superior mesenteric artery has been examined in anaesthetized rabbits in-vivo. Rabbits were fed with either standard or cholesterol-enriched diet for 24 weeks. Plasma lipids and changes in the endothelin content of plasma and vascular tissue were measured in the superior mesenteric artery and in the thoracic aorta. The functional severity of atherosclerosis was determined by examining vascular responses in the isolated thoracic aorta. The blood flow in the superior mesenteric artery was measured by transit-time flowmetry and drugs were injected through an intra-abdominal aortic catheter.

Acetylcholine (5, 10, 20 $\mu g\,kg^{-1}$) elicited dose-dependent, mesenteric vasodilation in normocholesterolaemic rabbits. In hypercholesterolaemic animals the response to acetylcholine was completely abolished and even became a vasoconstriction. Endothelin levels in plasma and in the vascular tissue were significantly elevated in hypercholesterolaemic animals compared with controls. Cromakalim at a dose of 3 $\mu g\,kg^{-1}$, elicited similar mesenteric vasodilation in hypercholesterolaemic and normocholesterolaemic animals.

These experiments show that the endothelium-dependent responses of the superior mesenteric artery to acetylcholine are functionally impaired by prolonged hypercholesterolaemia, that this altered vascular reactivity is associated with the elevation of endothelin levels in the circulation and in vascular tissues, and that in hypercholesterolaemia the mesenteric vasodilator effect of the K^+-channel opener cromakalim is entirely preserved, suggesting that severe hypercholesterolaemia does not depress the function of ATP-sensitive potassium channels in mesenteric vascular smooth muscle.

Endothelial cells play an important role in the control of vascular tone (Furchgott & Zawadzki 1980; Miller 1991; Vanhoutte et al 1991; Ralevic et al 1992; Rubányi 1993). Damage to or dysfunction of endothelial cells is widely regarded as a critical initiating factor in atherogenesis (Rubányi 1993). Changes in endothelial function can result from reduced synthesis or release of endothelium-dependent relaxing factors (EDRFs) or augmented synthesis or release of endothelium-dependent contracting factors (EDCFs), or both. The possibility of defective humoral or electrical coupling between the smooth muscle cells has also been suggested (Verbeuren & Herman 1988; Burnstock et al 1991; Rubányi 1993). The complex mechanisms by which hypercholesterolaemia and atherosclerosis impair the activity of EDRF remain elusive, although it is clear that endothelium-dependent relaxation is depressed throughout the arterial tree (Cohen 1995; Harrison & Ohara 1995). In animal models of atherosclerosis or hypercholesterolaemia impairment of endothelium-dependent relaxation to acetylcholine has been associated with relative preservation of endothelium-independent vasodilation (Verbeuren et al 1986a, b; Verbeuren & Herman 1988; Kolodgie et al 1990; Burnstock et al 1991). Although alterations in endothelium-dependent vasodilator activity are well established in atherosclerosis, few studies have dealt with the possible pathophysiological significance of endothelin, known to be the most potent EDCF (Lerman et al 1991; Patrignani et al 1992; Masaki 1993; Bacon et al 1995, 1996).

Previous studies have also suggested that the ATP-sensitive potassium channel (K_{ATP}) might be

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important in regulation of the vascular smooth muscle cell-membrane potential and tone, especially in the mesenteric vascular bed (McCarron et al 1991; Silberg & Breewen 1992; Edwards & Weston 1993; Mayhan 1993; Meisheri et al 1993; Pfrönder et al 1993). Whether atherosclerosis alters the functional responses of the mesenteric artery to K_{ATP}-channel activation is not known.

The aims of this study were:

To determine whether K_{ATP}-channel activation is decisively involved in the preserved endothelium-independent vasodilator activity of mesenteric vascular smooth muscle after long-term (24 weeks) hypercholesterolaemia in a rabbit model. The effects of cromakalim on the vascular reactivity of mesenteric arterial bed were therefore studied in-vivo.

To investigate whether the altered mesenteric vascular reactivity in hypercholesterolaemia, as assessed by the impaired vasodilator response to acetylcholine, is associated with elevation of the endothelin levels of the plasma and vascular tissues.

To analyse the physiological responsiveness of the isolated thoracic aorta to endothelium-dependent and -independent vasodilators.

Materials and Methods

Drugs and solutions

Acetylcholine chloride and phenylephrine hydrochloride were purchased from Sigma (St Louis, MO); fresh solutions were prepared daily. Stock solution of (±)-cromakalim (Beecham Pharmaceuticals, Betchworth, UK) was prepared in 96% ethanol and stored at −20°C for a week. Nitroglycerine (Nitrolingual) solution was obtained from G. Pohl-Boskamp, Hohenlockstedt, Germany and diluted with twice-distilled water. All concentrations indicated in the text and in figures are expressed as final concentrations. None of the substances was applied to the organ bath in a volume greater than 150 μL. Krebs-Henseleit solution consisted of (mmol L^{-1}): NaCl 120, KCl 4·2, $CaCl_2$ 1·5, $NaHCO_3$ 20, $MgCl_2$ 1·2, KH_2PO_4 1·2 and glucose 11.

Animals

All experiments in this study were approved by the Ministry of Science and Research of the Republic of Hungary and conducted in strict compliance with established professional and National Institutes of Health guidelines. Male New Zealand White rabbits, 820–1200 g, were obtained 1 week post-weaning from the Biocentre Services Unit in Szeged, Hungary. The animals were maintained individually in cages at constant temperature (23 ± 2°C) and humidity (55 ± 5%) under a 12-h dark–light cycle. All animals had free access to water and were in good health at the time of the study.

Induction of experimental hypercholesterolaemia

After an adaptation period of 1 week the animals were separated into 2 equal groups which were randomly assigned to 1 of 2 dietary regimens. Group 1 comprised controls fed a standard commercial chow containing eight vitamins (Lati Nutritional Biochemicals, Gödöllö, Hungary). Group 2 comprised hypercholesterolaemic animals fed a diet supplemented with 1% cholesterol and eight vitamins. The feeding regimen consisted of 40-g meals for the first week, 50-g meals for the next 4 weeks, 100-g meals for the next 12 weeks and 200-g meals for the final 7 weeks. During the experimental period the increase in body weight at constant rate for the hypercholesterolaemic rabbits was slightly less than that for the controls, resulting in final body weights of 3210 ± 158 g for controls and 3005 ± 114 g for the cholesterol-fed rabbits. Blood was drawn from the central ear artery at the beginning of the experiment and every 2 months thereafter. Finally, blood samples were collected at the beginning of surgery to monitor total plasma cholesterol and triglyceride levels.

Experimental preparation

At the end of the dietary period the rabbits were fasted for 24 h before the experiments but drinking water was still freely available. The animals were sedated with diazepam (10 mg kg^{-1}) and deeply anaesthetized with ketamine hydrochloride (10 mg kg^{-1}), given intravenously. Additional doses of ketamine hydrochloride (1 mg kg^{-1}) were administered to maintain anaesthesia.

The rectal temperature was maintained at 37–38°C by means of a heating pad. The mean systemic arterial blood pressure was determined continuously by use of a carotid catheter (PE 50) filled with heparinized (20 units mL^{-1}) isosmotic saline. The catheter tip was positioned in the ascending aorta and connected to a strain-gauge transducer (P 23 Db; Statham Instruments, Oxnard, CA). The transducer signals were amplified and recorded with a Gould transducer amplifier and a Hellige six-channel recorder. The heart rate was calculated from recordings of phasic arterial pressure. The left femoral vein was catheterized for intravenous delivery of drugs. After a midline laparotomy an appropriately sized, factory-calibrated ultrasonic transit-time flowmeter transducer (model 2 S24; Transonic Systems, Ithaca, NY) was implanted on

the trunk of the superior mesenteric artery (the splanchnic nerves and lymphatic channels crossing the superior mesenteric artery were preserved) and the blood flow was recorded with the ultrasonic transit-time shift technique (model T206; Transonic Systems, Ithaca, NY; Sumi et al 1987), which measures the net volume of blood flow ($mL\,min^{-1}$). The signals were monitored continuously as the pulsatile and mean flow on the recorder. In each of the experiments the electronic zero flow was equal to the mechanical zero flow obtained by arterial occlusion distal to the probe. The flowmeter was calibrated with saline and found to be linear $\pm 6\%$ in the range 0–550 $mL\,min^{-1}$. Drugs were injected into the abdominal aorta via a catheter (PC 350) advanced up the left common iliac artery into the abdominal aorta until its tip was located 4 mm proximal to the origin of the superior mesenteric artery. The position of the catheter tip was confirmed by manual compression of the aorta before each experiment. Between drug injections the catheter was filled with heparinized saline. After surgery the preparation was left to stabilize for 25 min, during which time a slow intravenous infusion of saline was administered.

Vascular conductance (C, $mL\,min^{-1}\,mmHg^{-1}$), calculated as mean blood flow divided by mean arterial blood pressure (Lautt 1989), was calculated before and during vasoactive drug or vehicle administration; the effect of the drug was expressed as a percentage of baseline conductance. Experimental conductance values were compared with the control conductance at the point of peak increase in mean blood flow.

Determination of plasma cholesterol and triglyceride levels

The lipid content was determined in plasma (10 mL) obtained from rabbits fasted overnight for at least 14 h. Plasma total cholesterol (Siedel et al 1983) and total triglyceride levels (Trinder 1969) were measured by means of an automatic analyser (Model 700 Chemistry System; Beckman, Miami, FL) using Boehringer Mannheim (Ingelheim, Germany) cholesterol and triglyceride kits. Cholesterol was measured with the CHOD-POD-PAP enzyme kit (where CHOD is cholesterin oxidase, POD is peroxidase and PAP is *p*-aminophenol) and triglycerides with the GPO-PAP enzymatic kit (where GPO is glycerol phosphoxidase).

Determination of plasma and vascular tissue endothelin levels

One day before the study and after the treatment endothelin levels were measured for the different groups. Plasma- and tissue-endothelin concentrations were measured by radioimmunoassay. Plasma samples for endothelin determination were collected in ice-chilled EDTA polystyrene tubes, centrifuged at 4°C, and stored at −25°C until determination. Endothelins were extracted from plasma by absorption on to prewashed SepPak C_{18} cartridges (Waters Millipore, Watford, UK) by slow passage of 5-mL plasma samples using a vacuum manifold (Univac; Uniequip, Martinsried, Germany). Eluates were concentrated by means of a vacuum centrifuge (Univac; Uniequip), resuspended, and then quantitatively determined with a sensitive radioimmunoassay (Biomedica, Vienna, Austria). Deeply frozen rabbit artery specimens (thoracic aorta and superior mesenteric artery rings 3 × 3 mm) were quickly weighed. The tissue samples (20–50 mg) were placed in boiling water (1 mL) and maintained at 100°C for 5 min. The mixtures were then homogenized and centrifuged ($3000\,rev\,min^{-1}$). Finally the supernatant was stored at −70°C before assay (2 weeks). Endothelin content was expressed in units of $fmol\,mg^{-1}$ frozen wet weight of tissue (Németh et al 1992).

Histology

Adjacent sections of thoracic aorta and superior mesenteric artery from all animals were stained with haematoxylin and eosin to enable histological examination.

In-vitro measurement of isometric tension in rabbit thoracic aorta

The thoracic aorta was carefully removed, dissected free from adjoining connective tissue and immersed in a room-temperature bath of Krebs-Henseleit solution. The aorta was then cut into 5-mm rings and suspended in 100-mL organ chambers filled with Krebs-Henseleit solution (oxygenated with 95% O_2–5% CO_2; pH 7·4 at 37°C).

Rings were mounted in a 2-mL recording chamber containing Krebs-Henseleit solution for recording of isometric tension (Type F30, Hugo Sachs Elektronic, Germany). A resting tension of 10 mN was applied to the tissues which were incubated in this condition for 45 min. Mechanical responses of arterial rings were displayed by means of a pen recorder (Type 175, Kutesz, Hungary).

Endothelium-intact and atherosclerotic ring preparations were exposed to 0·25 μM phenylephrine. When the phenylephrine-induced steady-state tone developed we investigated the vascular effects of endothelium-dependent acetylcholine (0·025–1·6 μM) and substance P (0·62–77·6 nM) and of the endothelium-independent vasodilators nitroglycerine

(1–1000 nM) and cromakalim (0·06–4 μM). Drugs were added cumulatively.

In-vivo experimental protocol

Experiments were performed on three groups. In Group I the cardiovascular effects of acetylcholine were determined in control rabbits (n = 14) and in hypercholesterolaemic rabbits (n = 14). Dose–response curves were obtained during administration of acetylcholine at successive doses of 5, 10 and 20 $\mu g\,kg^{-1}$. Doses were administered at least 15 min apart to avoid tachyphylaxis. In Group II the cardiovascular effects of cromakalin were determined in control rabbits (n = 6). Dose–response curves were obtained during administration of cromakalim at successive doses of 1, 3 and 9 $\mu g\,kg^{-1}$. Periods of 35 min were allowed between the dose–response experiments. Similarly, hypercholesterolaemic rabbits (n = 8) were given intra-aortic injections of 3 $\mu g\,kg^{-1}$ cromakalim. Group III was used to study the reproducibility of the cardiovascular effects of cromakalim. Cromakalim (3 $\mu g\,kg^{-1}$) (n = 3) or vehicle (n = 3) was administered to control rabbits and haemodynamic parameters were monitored. Animals were re-challenged 40 min after initial application of drug or vehicle.

Data analysis

Data are presented as means ± s.e. The probability that a difference was significant was determined by analysis of variance within each experimental group, in which each rabbit served as its own control. The constancy of systemic haemodynamics during intervention was assessed in a repeated measures analysis of variance. Significant differences between results obtained before and after administration of drugs were assessed by use of Student's paired *t*-test. Repeated measurements were compared by use of the Bonferroni correction for multiple comparisons. Statistical significance was defined as $P < 0.05$. Relaxations to the vasoconstrictor and vasodilator agents are expressed in millinewtons (mN). Values are given as means ± s.e.m. Statistical evaluation of the data was performed by Student's *t*-test for unpaired observations. Significance was accepted at the 95% confidence interval ($P < 0.05$). EC50 values (the dose having half the maximum effect) and maximum relaxations were calculated by fitting the exponential equation:

$$A/(1 + \exp(b \times (x - c))) \quad (1)$$

Results

The relationship between amount of diet-induced hypercholesterolaemia and changes in the biochemical parameters of the rabbit

Time-dependent changes of total plasma cholesterol and triglyceride levels. Table 1 summarizes the lipid profiles of the two experimental groups at baseline and after 2, 4 and 6 months of diet. There was no difference between the baseline values of the experimental groups. Plasma cholesterol and triglyceride levels gradually increased, from 2 ± 0·5 to 267 ± 25 and from 0·6 ± 0·1 to 36 ± 7 mmol L^{-1}, respectively, in rabbits (Group 2) fed a 1% cholesterol diet for 6 months. The results were significantly ($P < 0.001$) different from those for Group 1 and from baseline values.

Changes in plasma and arterial tissue endothelin levels. Samples of thoracic aorta and superior mesenteric artery containing raised atherosclerotic plaques or fatty streaks were obtained from the hypercholesterolaemic group. Histologically normal arteries were taken from age-matched control group. Table 2 illustrates the changes in the plasma and arterial tissue levels of endothelin in the two groups. There was no significant difference between the baseline values of the experimental groups. The mean plasma and arterial tissue levels of endothelin were significantly ($P < 0.001$) elevated in the hypercholesterolaemic group compared with the control group.

Table 1. The lipid profiles of the experimental groups.

	Group 1: normal diet (n = 26)		Group 2: cholesterol diet (n = 22)	
	Cholesterol (mM)	Triglyceride (mM)	Cholesterol (mM)	Triglyceride (mM)
Baseline	2 ± 0·3	0·6 ± 0·1	2 ± 0·5	0·6 ± 0·1
2 month diet	4 ± 0·5	1 ± 0·4	64 ± 6***	6 ± 1***
4 month diet	5 ± 0·6	1 ± 0·5	115 ± 14***	15 ± 6***
6 month diet	5 ± 0·7	2 ± 0·7	267 ± 25***	36 ± 7***

Values are means ± s.e. of results from 26 control or 22 hypercholesterolaemic animals. ***$P < 0.001$, significantly different from baseline result or result from group receiving normal diet (Bonferroni correction).

Table 2. Plasma and arterial tissue levels of endothelin in the experimental groups.

	Plasma (fmol mL^{-1})	Aorta (fmol mg^{-1})	Superior mesenteric artery (fmol mg^{-1})
Group 1: Normal diet			
Baseline	3·6 ± 0·4	0·1 ± 0·06	0·1 ± 0·08
6 Month diet	4·4 ± 0·3	0·2 ± 0·05	0·3 ± 0·06
Group 2: 1% Cholesterol diet			
Baseline	2·8 ± 0·3	0·1 ± 0·04	0·1 ± 0·08
6 Month diet	7·8 ± 2·1***	0·5 ± 0·1***	0·8 ± 0·1***

Values are means ± s.e. of results from 26 control or 22 hypercholesterolaemic animals. ***$P < 0.001$, significantly different from baseline result or result from group receiving normal diet (Bonferroni correction).

Endothelium-dependent and independent relaxations in rabbit isolated aorta. In control (n = 9) and cholesterol-fed (n = 11) rabbits phenylephrine (0·25 μM) evoked contractions of 39·9 ± 3·9 and 19·6 ± 1·7 mN, respectively ($P < 0.001$). Concentration-dependent relaxations to acetylcholine and substance P with calculated EC50 values of 0·068 ± 0·011 μM and 0·861 ± 0·09 nM, respectively, were measured for rings from animals maintained on normal diet (Figures 1A, B). For aortic preparations obtained from rabbits fed with cholesterol-rich chow the maximum relaxation induced by acetylcholine was significantly lower than normocholesterolaemic values (Figure 1A). The EC50 value for acetylcholine was significantly enhanced in this group compared with the control (0·203 ± 0·059 μM compared with 0·068 ± 0·011 μM, respectively, $P < 0.05$) and substance P completely abolished phenylephrine-induced tone (Figure 1B). The effect of long-term hypercholesterolaemia on endothelium-independent vaso-relaxation is depicted in Figures 2A, B. The calculated EC50 for nitroglycerine was increased from 36·3 ± 5·2 nM to 107·1 ± 12·1 nM ($P < 0.001$); that for cromakalim was not significantly affected in the hypercholesterolaemic group (3·51 ± 0·93 μM for normocholesterolaemic compared with 3·27 ± 1·1 μM, for hypercholesterolaemic). The maximum response of the hypercholesterolaemic blood vessels to the endothelium-independent relaxants was reduced, but to a smaller extent than to the endothelium-dependent relaxants. The relative magnitude of maximum depression was 90% for acetylcholine and 100% for substance

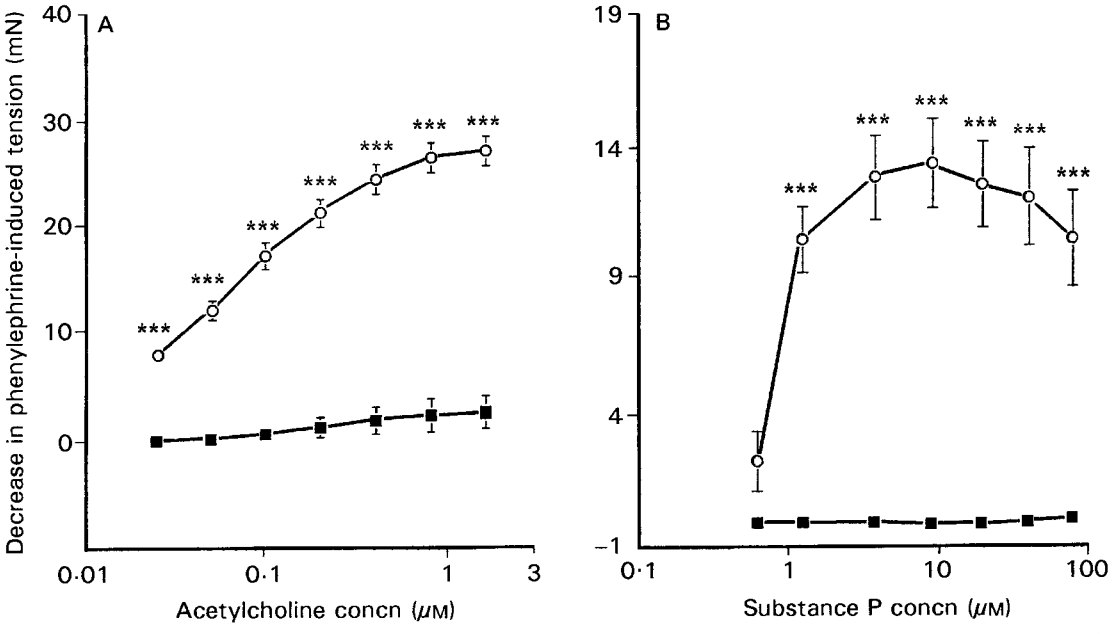

Figure 1. Effects of endothelium-dependent vasodilators (acetylcholine, A; substance P, B) on the tone of phenylephrine-precontracted thoracic aorta isolated from control (○, n = 9) and atherosclerotic (■, n = 11) rabbits. ***$P < 0.001$, significantly different from control result.

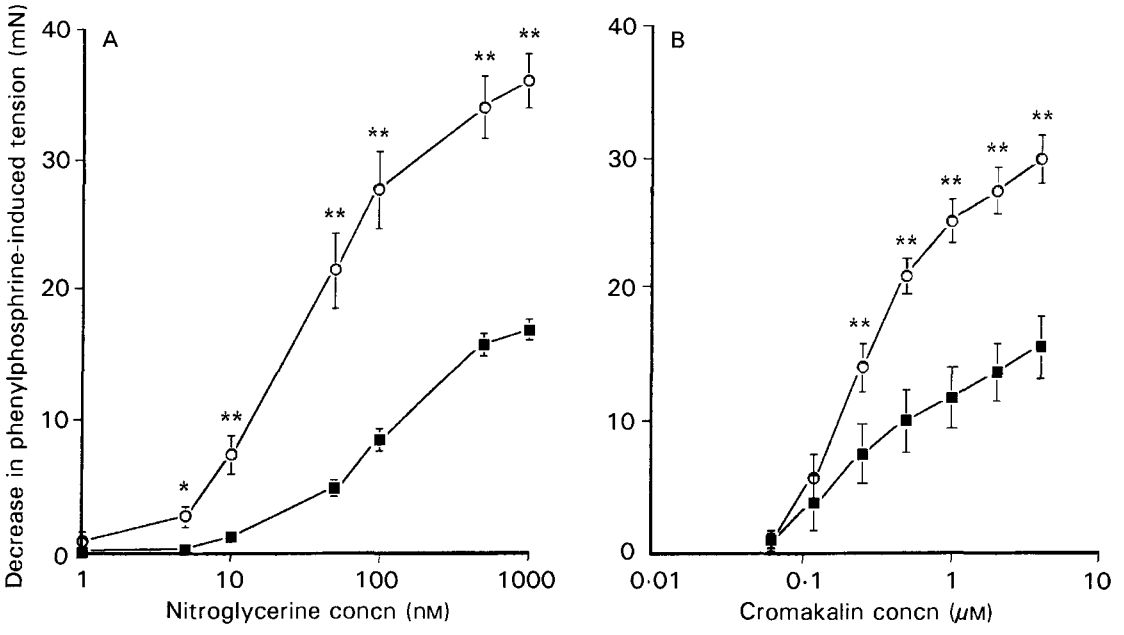

Figure 2. Effects of endothelium-independent vasodilators (nitroglycerine, A; cromakalim, B) on the tone of phenylephrine-precontracted thoracic aorta isolated from control (○, n = 6) and atherosclerotic (■, n = 6) rabbits. $^{*}P < 0.05$, $^{**}P < 0.01$, significantly different from control result.

P whereas relaxations by nitroglycerine and cromakalim decreased by 52% and 47% compared with normocholesterolaemic values. These results are indicative of severe depression of endothelial function and partial preservation of the relaxing capacity of aortic smooth muscle in advanced atherosclerosis.

Cardiovascular effects of acetylcholine. Table 3 lists the dose–response data obtained after intra-aortic administration of acetylcholine to rabbits. The baseline haemodynamic values in the control and hypercholesterolaemic animals did not differ significantly—superior mesenteric artery blood flow values averaged 96 ± 8 mL min^{-1} in controls, 88 ± 6 mL min^{-1} in hypercholesterolaemic animals.

Injection of acetylcholine at doses of 5 and 10 μg kg^{-1} to control animals caused immediate dose-dependent, significant increases in superior mesenteric artery blood flow. The vascular responses peaked 2 ± 0.4 min after administration of acetylcholine and returned to the control level after 4 ± 0.5 or 6 ± 1.2 min. The mean arterial blood pressure and heart rate did not change significantly with increasing doses of acetylcholine from 5 to 10 μg kg^{-1}. When acetylcholine was administered at a dose of 20 μg kg^{-1} there was a transient drop in mean arterial blood pressure, accompanied by a decrease in heart rate and dose-dependent increases in the perfusion of the superior mesenteric artery. The mean arterial blood pressure and heart rate returned to baseline within 4 min and the blood-flow changes returned to baseline within 9 ± 1.4 min after this dose of acetylcholine. Under the same experimental conditions in hypercholesterolaemic rabbits the acetylcholine (5 or 10 μg kg^{-1}) -induced dilation of the superior mesenteric artery was attenuated compared with that in the controls ($P < 0.001$). Furthermore, 20 μg kg^{-1} acetylcholine immediately elicited significant decreases in the perfusion of the superior mesenteric artery ($P < 0.001$). The vasoconstriction of the superior mesenteric artery peaked 2 ± 0.8 min after acetylcholine administration and returned to the control level after 10 ± 1.3 min. The absolute values at the beginning and end of the experiment did not differ significantly between the control and hypercholesterolaemic animals.

Cardiovascular effects of cromakalim. Table 4 illustrates the haemodynamic effects of increasing doses of cromakalim in control rabbits. Intra-aortic administration of cromakalim at doses of 1, 3 and 9 μg kg^{-1} induced significant ($P < 0.001$) dose-dependent increases in superior mesenteric artery blood flow accompanied by significant ($P < 0.001$) dose-dependent reductions in mean arterial blood pressure and increases in heart rate.

Table 3. Haemodynamic effects of acetylcholine in control and hypercholesterolaemic rabbits.

	Acetylcholine ($\mu g\ kg^{-1}$)		
	5	10	20
Control rabbits (n = 14)			
Mean arterial pressure (mm Hg)			
before	104 ± 5	108 ± 10	102 ± 8
after 2 min	106 ± 8	100 ± 12	86 ± 11*
Heart rate (beats min^{-1})			
before	210 ± 12	220 ± 7	225 ± 14
after 2 min	216 ± 7	215 ± 9	200 ± 7*
Superior mesenteric artery conductance (mL min^{-1} $mmHg^{-1}$)			
before	0·53 ± 0·04	0·52 ± 0·06	0·50 ± 0·05
after 2 min‡	38 ± 5***	79 ± 8***	184 ± 15***
Hypercholesterolaemic rabbits (n = 14)			
Mean arterial pressure (mm Hg)			
before	95 ± 6	90 ± 10	88 ± 9
after 2 min	86 ± 8	82 ± 12	62 ± 11*
Heart rate (beats min^{-1})			
before	214 ± 12	221 ± 14	210 ± 15
after 2 min	216 ± 9	217 ± 10	176 ± 9***
Superior mesenteric artery conductance (mL min^{-1} $mmHg^{-1}$)			
before	0·48 ± 0·06	0·46 ± 0·05	0·45 ± 0·07
after 2 min†	6 ± 3	6 ± 5	−52 ± 8***

Values are means ± s.e. Comparison is made before addition of acetylcholine and 2 min after. *$P < 0.05$, ***$P < 0.001$, significantly different from pre-drug values (analysis of variance). †Data are presented as % changes in baseline.

The haemodynamic effects of cromakalim differed from those of acetylcholine in two respects—the onset of vasorelaxation with cromakalim was slower than that with acetylcholine (1·6 ± 0·5 min), and the vasorelaxant effect of cromakalim was more persistent than that of acetylcholine. Intra-aortic administration of 3 $\mu g\ kg^{-1}$ cromakalim, which was found to result in almost 50% relaxation of the superior mesenteric artery in control animals, to hypercholesterolaemic rabbits under the same experimental conditions evoked virtually identical haemodynamic responses to those for the controls. The cardiovascular response was a significant fall in mean arterial blood pressure, an increase in heart rate and an increase in the perfusion of the superior mesenteric artery ($P < 0.001$). The time-courses of the haemodynamic responses were similar, including a lag of 2 ± 0·8 min before the beginning of the responses. In the reproducibility study in control rabbits, repeated administration of 3 $\mu g\ kg^{-1}$ cromakalim after 40 min evoked nearly identical cardiovascular effects (data not shown). In separate control experiments, the mean arterial blood pressure, heart rate and superior mesenteric artery blood flow was not affected by intra-aortic injection of saline or vehicle and the recorded parameters were stable after 2 h (data not shown).

Discussion

The experiments were designed to examine the effects of prolonged hypercholesterolaemia on the vascular reactivity of the mesenteric vascular bed. To the best of our knowledge this is the first comparative in-vivo study to report altered vascular function in the mesenteric vascular bed of rabbits with hypercholesterolaemia. There are four important new findings:

induction of hypercholesterolaemia in rabbits results in an attenuated vasodilatory response to systemically administered acetylcholine in the mesenteric vascular beds;

there is no difference in-vivo between the cromakalim-induced vasorelaxation response of control and hypercholesterolaemic rabbits;

induction of hypercholesterolaemia results in elevated circulating plasma endothelin and in enhanced vascular tissue concentration of endothelin in rabbits;

Table 4. Haemodynamic effects of cromakalim in control and hypercholesterolaemic rabbits.

	Cromakalim ($\mu g\ kg^{-1}$)		
	1	3	9
Control rabbits n = 6			
Mean arterial pressure (mm Hg)			
before	100 ± 6	90 ± 9	96 ± 11
after 10 min	80 ± 4*	59 ± 11***	40 ± 14***
duration (min)	12 ± 5	26 ± 10	38 ± 12
Heart rate (beats min^{-1})			
before	212 ± 10	218 ± 14	220 ± 12
after 10 min	224 ± 6*	242 ± 11***	250 ± 14***
duration (min)	10 ± 4	22 ± 9	44 ± 16
Superior mesenteric artery conductance (mL min^{-1} $mmHg^{-1}$)			
before	0·46 ± 0·02	0·48 ± 0·04	0·52 ± 0·06
after 10 min‡	28 ± 4*	69 ± 10***	90 ± 14***
duration (min)	18 ± 4	34 ± 11	54 ± 12
Hypercholesterolaemic rabbits n = 8			
Mean arterial pressure (mm Hg)			
before		108 ± 7	
after 10 min		54 ± 13***	
duration (min)		28 ± 8	
Heart rate (beats min^{-1})			
before		209 ± 12	
after 10 min		248 ± 9***	
duration (min)		26 ± 6	
Superior mesenteric artery conductance (mL min^{-1} $mmHg^{-1}$)			
before		0·58 ± 0·03	
after 10 min‡		52 ± 12***	
duration (min)		29 ± 7	

Values are means ± s.e. Comparison is made before addition of cromakalim and 10 min after. *$P < 0.05$, ***$P < 0.001$, significantly different from pre-drug values (analysis of variance). ‡Data are presented as Δ% changes in baseline.

the in-vitro study supports our in-vivo findings that hypercholesterolaemia results in reduced vasodilation in response to endothelial-dependent substances, such as acetylcholine and substance P, whereas the response to potassium-channel activation remains relatively preserved in the thoracic aorta.

In this study we demonstrated a very severe diminution of endothelial function as assessed by acetylcholine- and substance P-induced vasorelaxation. The response to acetylcholine was completely abolished and even a high dose induced puzzling mesenteric vasoconstriction in hypercholesterolaemic animals. Contraction of aortic rings to α_1-adrenergic stimulation and vascular response to endothelium-independent vasodilators were also found to be significantly reduced compared with control values. Other authors have demonstrated variable effects or no change in endothelium-independent functions of the rabbit aorta (Henry & Yokoyama 1980; Kolodgie et al 1990; Burnstock et al 1991), although those studies used less severe atherosclerotic animals. In the current study, a very high cholesterol level after six months of atherogenic diet (an average of 267 mmol L^{-1}) might have resulted in functional impairment not only of the endothelium but also the smooth-muscle cells. The relative preservation of the endothelium-independent vasorelaxation by nitroglycerine and cromakalim support the functional importance of the cyclic guanosine 5′-phosphate signalling system and electro-mechanical coupling of the smooth muscle in maintaining the response of the aortic tissue to pharmacological stimuli.

Cromakalim was found to be a potent relaxant of the superior mesenteric artery. More importantly, it exerted similar vasodilatory responses in control and hypercholesterolaemic animals. The finding that the responses to cromakalim were not altered suggests that the function of mesenteric vascular smooth muscle K_{ATP} channels is preserved in hypercholesterolaemic animals. These results might have therapeutic importance in disease states with altered endothelial function.

Another important finding in this study was the evidence that endothelin levels of plasma and vascular tissues were significantly elevated in hypercholesterolaemic animals compared with controls. This finding supports previous studies which suggested an important role of endothelin as a marker for arterial vascular disease in atherosclerosis in man (Lerman et al 1991; Bacon et al 1995, 1996). The vascular effects of endothelin and its potential source within diseased arteries make it a good candidate as a factor promoting vasoconstriction in this model of long-term hypercholesterolaemia. Verification of this hypothesis might require selective pharmacological modification of local vascular endothelin synthesis or action.

In conclusion, we have shown that the endothelium-dependent responses of the mesenteric artery to acetylcholine are impaired in hypercholesterolaemic rabbits. In contrast, the dilation of the mesenteric artery in response to cromakalim is entirely preserved. The results suggest that elevation of circulating and mesenteric arterial tissue levels of endothelin might significantly contribute to the pathomechanism of hypercholesterolaemia-induced vascular dysfunction. The possible beneficial functional effects of pharmacological manipulation of local vascular endothelin production or stimulation of ATP-sensitive potassium channels in hypercholesterolaemia-induced mesenteric endothelial dysfunction require further study.

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