Reduced endothelium-dependent relaxation at enhanced NO release in hearts of hypercholesterolaemic rabbits

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1 Langendorff hearts, perfused at constant volume, were prepared from rabbits fed a cholesterol-enriched diet for 4 months. Coronary perfusion pressure and nitric oxide (NO) release (oxyhaemoglobin technique) into the coronary effluent were measured continuously. Prostacyclin (PGI₂) in the effluents was determined by radioimmunoassay (6-oxo-PGF_{1α}).

2 Basal NO release was not different between control and hypercholesterolaemic rabbits. However, the coronary vasculature of hypercholesterolaemic rabbits showed a considerably (> 50%) reduced endothelium-dependent relaxation in response to short-term (3 min) infusion of bradykinin (50 nM) and substance P (50 nM) ($P < 0.05$, $n = 8-9$). Under these conditions, NO release into the vessel lumen was increased, by 26%, in hypercholesterolaemic hearts ($P < 0.05$, $n = 8-9$). N^G-nitro-L-arginine (L-NOARG, 30 μM) significantly attenuated both bradykinin-induced NO formation and vessel relaxation in control hearts but only NO release in hypercholesterolaemia. L-Arginine (200 μM) restored the response to that before L-NOARG but did not improve the reduced endothelium-dependent relaxation in cholesterol-fed rabbits.

3 Superoxide dismutase (10 U ml⁻¹) significantly improved vessel relaxation without changing the hypercholesterolaemia-related coronary dysfunction. Vasodilatation in response to exogenous NO donors (linsidomine) was diminished in hypercholesterolaemia as compared to controls.

4 Basal PGI₂ release was unchanged in hypercholesterolaemic hearts. There was a tendency in these hearts for greater PGI₂ formation after stimulation by substance P and bradykinin ($P \geq 0.05$). The coronary relaxation to iloprost was unchanged.

5 The data demonstrate impaired endothelium-dependent relaxation of coronary arterial resistance vessels in hypercholesterolaemia. This diminished vascular response was not due to reduced NO generation but probably a reduced action of released NO, either by accelerated degradation and/or disturbed signal transduction pathways to vascular smooth muscle cells. There was no significant change in PGI₂ related pathways of vasomotor control in hypercholesterolaemia.

Keywords: EDRF; nitric oxide; hypercholesterolaemia; L-arginine; superoxide dismutase (SOD); prostacyclin (PGI₂)

Introduction

Local control of vessel tone is mainly exerted via generation and release of chemical mediators. Two different types of endothelium-derived vasodilators have received particular attention: nitric oxide (NO) and prostacyclin (PGI₂). Stimulation of endothelial function by hormones or mechanical stress results in increased generation of these compounds and subsequent vessel relaxation.

This physiological interaction between vascular endothelium and smooth muscle may be disturbed under conditions of endothelial dysfunction. For example, hypercholesterolaemia results in diminished endothelium-dependent relaxation. This observation, originally reported in man by Ludmer *et al.* (1986) suggested reduced formation of endothelium-derived relaxing factor (EDRF) and was confirmed in several later studies (Verbeuren *et al.*, 1986; Osborne *et al.*, 1989; Kappagoda *et al.*, 1990; Sellke *et al.*, 1990; Tagawa *et al.*, 1991). However, many of these studies were bioassay-type experiments where release of EDRF, i.e. NO, was measured in terms of vessel relaxation. Any reduced vessel relaxation could also have been caused by a more rapid degradation of the unstable mediator and/or reduced action on the vascular smooth muscle cells. Thus, the question whether reduced endothelium-dependent relaxation in hypercholesterolaemia is due to reduced mediator formation or reduced action remains unanswered.

A recent investigation by Minor *et al.* (1990), describing enhanced generation of nitrogen oxides in aortic tissue of hypercholesterolaemic rabbits gives a first hint that vascular

NO formation might not always be reduced in hypercholesterolaemia.

These observations resemble earlier findings on prostacyclin when the original studies described reduced PGI₂ formation in vessels of hypercholesterolaemic rabbits (Gryglewski *et al.*, 1978) while later work demonstrated enhanced vascular PGI₂ production in both animal (Beetens *et al.*, 1986) and human studies (FitzGerald *et al.*, 1984). Thus, the question arises of whether the formation of both mediators is under control of common denominators, such as hypercholesterolaemia-related cytokine production that may up-regulate enzyme proteins at the message level (Pommerantz *et al.*, 1993).

The present study was designed to measure directly formation and action of NO and PGI₂ in the coronary microcirculation of the hypercholesterolaemic rabbit. Specifically, the following questions were studied: (i) Does hypercholesterolaemia impair endothelium-dependent NO and PGI₂ formation in the plaque-free coronary microcirculation as opposed to the conventional studies on plaque-containing aortic tissue, (ii) is such impairment correlated to changes in coronary vessel tone, i.e. coronary vascular resistance, and (iii) does any impairment of vessel relaxation involve an altered vessel reactivity towards exogenous mimetics, such as linsidomine (SIN-1) or iloprost.

Methods

Hypercholesterolaemia

New Zealand White rabbits (1800–2000 g initial weight; $n = 8$) were fed for 12 weeks a standard laboratory diet,

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supplemented with 0.5% cholesterol and 3% olive oil. This was followed by 1% cholesterol and 3% olive oil for further 4 weeks. Age matched control animals ($n = 9$) were fed a cholesterol-free standard diet for 16 weeks. A blood lipid analysis was performed at the end of the feeding period in all animals. Some hearts were also investigated microscopically for plaque development in the coronary circulation. No overt atherosclerotic plaque formation was detected in the coronary circulation (not shown).

Heart preparation

At the end of the feeding period, the animals were killed by a blow to the head. The hearts were rapidly excised and perfused with Krebs-Henseleit-buffer (pH 7.4; 37°C) at a constant volume of 40 ml min⁻¹ according to the Langendorff-technique. The buffer was supplemented with oxyhaemoglobin (3 µM) and equilibrated with 95% O₂ + 5% CO₂ by the use of a membrane oxygenator (Eschweiler, Kiel, Germany). The hearts were beating spontaneously.

Measurements

Actively developed left ventricular systolic pressure (LVP, balloon technique) and coronary perfusion pressure (CPP) were monitored continuously by Statham transducers and recorded on a multichannel recorder.

NO generation was measured by the oxyhaemoglobin technique as previously described (Schrör *et al.*, 1991; Woditsch & Schrör, 1992). Briefly, the coronary effluent passed a flow-through cuvette which was inserted in a dual-wavelength spectrophotometer (Aminco DW-2000, Colora, Lorch, Germany). NO release into the vessel lumen was calculated from the photometric signal by determination of the area under the curve (AUC) of the amount above basal levels induced by vasodilator infusion. The detection limit of the method was 0.3 nM.

Prostacyclin (PGI₂) was measured in aliquots of the coronary effluent by radioimmunoassay of the stable hydrolysis product 6-oxo-PGF_{1α}. The detection limit was 10 pg ml⁻¹. PGI₂ formation was also calculated as total amount released from the area under the curve (AUC).

Vasodilator infusion

All vasodilators were administered as short-term (3 min) infusion directly into the coronary inflow tract by a perfusion pump (Braun Melsungen, Germany). The endothelium-dependent vasodilators, carbamoylcholine (0.1 µM), bradykinin (0.05 µM) and substance P (0.05 µM) were also used. These concentrations were previously found to be submaximal and equieffective with respect to release of NO and coronary vasodilatation (Woditsch & Schrör, 1992). As an endothelium-independent PGI₂-mimetic, iloprost (0.1 µM) was used, and as an NO-mimetic, linsidomine (SIN-1, 5 µM). SIN-1 has previously been shown in this model to release NO spontaneously in the perfusion buffer at vasoactive concentrations (Schrör *et al.*, 1991).

The NO synthase inhibitor N^G-nitro-L-arginine (L-NOARG, 30 µM), the NO precursor L-arginine (L-Arg, 200 µM) and superoxide dismutase (SOD, 10 u × ml⁻¹) were added to the perfusion buffer during the experiment (see Experimental protocol).

Substances and solutions

Bovine haemoglobin, N^G-nitro-L-arginine (L-NOARG), bradykinin, substance P, carbamoylcholine and superoxide dismutase (bovine) were purchased from Sigma (Deisenhofen, Germany). Iloprost (Schering AG, Berlin/Bergkamen, Germany) and linsidomine (SIN-1, Cassella-Riedel, Frankfurt/M., Germany) were kindly provided by the respective

manufacturers. The other chemicals were obtained from Merck (Darmstadt, Germany).

L-NOARG was dissolved in hydrochloric acid (0.1 mM) and further diluted in Krebs-Henseleit-buffer. Fresh solutions of SIN-1 were prepared 15 min prior to use. Stock solutions of substance P were prepared in acetic acid (10 mM) and further diluted in Krebs-Henseleit-buffer. The other compounds were dissolved in Krebs-Henseleit-buffer.

Experimental protocol

Figure 1 demonstrates the experimental protocol. Experiments were started after a 30 min equilibration period, when stable conditions of CPP, heart rate and LVP were obtained (min 0). In contrast to the other vasodilators, the relaxations induced by carbamoylcholine and SIN-1 were inhibited by oxyhaemoglobin. These vasodilators were tested before the perfusion buffer was supplemented with oxyhaemoglobin, and NO formation by carbamoylcholine and SIN-1 could not be measured. Then (min 20), oxyhaemoglobin (3 µM) was added to the buffer and bradykinin, substance P, and iloprost were infused for 3 min each. At the 70th min of the experiment, L-NOARG was added to the perfusion buffer, followed by another infusion of bradykinin. The perfusion was then changed to Krebs-Henseleit-buffer containing L-arginine (min 115). After a short equilibration period, bradykinin was again infused for 3 min and the perfusion was continued until min 160. Additionally, in some experiments at the 60th min bradykinin was infused in the presence of SOD (10 u ml⁻¹).

Before, during and after each vasodilator infusion (0, 1, 2, 3, 5 and 10 min after start of infusion) aliquots of the effluent were taken for determination of 6-oxo-PGF_{1α}. NO formation was measured continuously as described above.

Statistics

The data are mean ± s.e.mean of n determinations. Statistical analysis was performed by the two-tailed t test. P levels of <0.05 were considered significant.

Results

Plasma-cholesterol

Total plasma cholesterol levels were enhanced after cholesterol feeding, from 0.7 ± 0.1 mM (27 ± 4 mg dl⁻¹) in the control group to 28.5 ± 2.0 mM (1102 ± 77 mg dl⁻¹) ($P < 0.01$).

Cardiac and coronary function

Left ventricular systolic pressure (LVP) and heart rate were not significantly different between cholesterol-fed and control animals at any time of the experiment. For example, LVP was 63 ± 4 mmHg in the controls and 69 ± 5 mmHg in cholesterol-fed animals ($P > 0.05$, $n = 8-9$). The respective data for heart rate were 185 ± 5 beats min⁻¹ vs. 180 ± 7 beats min⁻¹ ($P > 0.05$, $n = 8-9$). The initial (time 0) coronary perfusion pressure (CPP) was also not different, amounting to 46 ± 3 mmHg in controls and 43 ± 3 mmHg in the cholesterol-fed group ($P > 0.05$, $n = 8-9$).

Figure 1 demonstrates alterations of the CPP during the acute experiment. Inhibition of luminally released NO by oxyhaemoglobin slightly enhanced basal levels of CPP. Inhibition of NO synthase by L-NOARG resulted in a significant increase of CPP. This increase was not reversed by subsequent addition of L-Arg. Apparently identical changes were obtained in hearts of hypercholesterolaemic rabbits and control animals. Because of this significant increase in CPP by L-NOARG, all relaxations induced by short-term infusion of vasodilators are expressed as % decrease of CPP from the

actual baseline to allow direct comparisons of the different treatments.

Vasodilator infusion

Figure 2 exemplifies the effect of vasodilator infusion by two original tracings, of a control and a hypercholesterolaemic heart, respectively. All vasodilators caused a significant decrease in CPP that returned rapidly to baseline level after the infusion was stopped. This vasodilatation in response to endothelium-dependent stimuli, i.e. carbamoylcholine, bradykinin, and substance P was markedly (by > 50%) reduced in hearts of hypercholesterolaemic rabbits. There was also a significantly reduced relaxation in response to SIN-1 (5 μ M)

Figure 1 Changes in the coronary perfusion pressure (CPP) (mmHg) in Langendorff hearts of control rabbits (●) and hearts of cholesterol-fed animals (▲) during the standard experimental protocol. Initial buffer perfusion for 20 min is followed by perfusion with oxyhaemoglobin (3 μ M). Additional perfusion with N^G-nitro-L-arginine (L-NOARG, 30 μ M) and L-arginine (L-Arg, 200 μ M) is started at time 70 and 115 min, respectively. The data are mean $\pm$ s.e. mean of $n = 8-9$ experiments. There were no significant differences ($P > 0.05$) between control and cholesterol treatment at the same time points. * $P < 0.05$ by comparing 80 min (L-NOARG) with the time before (70 min).

in hypercholesterolaemia ($P < 0.05$, $n = 8-9$), while the vasodilatation induced by iloprost remained unchanged. The data of all experiments are summarized in Figure 3.

Generation of nitric oxide (NO)

Basal release of NO into the coronary perfusate was not different between the two groups of hearts: 1.57 ± 0.21 nM min⁻¹ in controls and 1.40 ± 0.23 nM min⁻¹ in hearts of cholesterol-fed animals ($P > 0.05$, $n = 8-9$).

Infusion of bradykinin and substance P into the coronary inflow tract of control hearts stimulated NO release by 2.62 ± 0.17 and 2.22 ± 0.21 nM, respectively. In hearts of hypercholesterolaemic rabbits, this stimulated NO release was significantly higher, amounting to 3.30 ± 0.18 nM with bradykinin and 2.80 ± 0.16 nM with substance P. This corresponded to a 26% increase of NO release in hypercholesterolaemic hearts in both cases ($P < 0.05$, $n = 8-9$) (Figure 4).

Generation of PGI₂

Basal release of 6-oxo-PGF_{1 α} was not different between the two groups of hearts. For example, at 20 min, basal release of 6-oxo-PGF_{1 α} was 20 ± 5 pM min⁻¹ in hearts of control rabbits and 21 ± 4 pM min⁻¹ in hearts of cholesterol-fed animals ($P > 0.05$, $n = 8-9$).

Both bradykinin and substance P stimulated 6-oxo-PGF_{1 α} release into the coronary effluent of control hearts by 0.44 ± 0.09 and 1.24 ± 0.20 nM, respectively. In hearts of hypercholesterolaemic rabbits, this release was slightly enhanced, amounting to 0.99 ± 0.33 nM for bradykinin and 1.47 ± 0.24 nM for substance P ($P \geq 0.05$, $n = 8-9$). These data are summarized in Figure 4. This stimulated 6-oxo-PGF_{1 α} generation was not significantly altered by subsequent treatment with L-NOARG or L-Arg (Table 1).

Effects of N^G-nitro-L-arginine (L-NOARG)

Inhibition of endogenous NO formation by L-NOARG was associated with a considerable increase in basal CPP, by

Figure 2 Original tracing of left ventricular systolic pressure (LVP), coronary perfusion pressure (CPP) and release of nitric oxide (NO) and prostacyclin (6-oxo-PGF_{1 α}) into the coronary effluent of a normocholesterolaemic control rabbit and a hypercholesterolaemic rabbit. The black lines below mark the infusion of carbamoylcholine (CCh, 0.1 μ M), linsidomine (SIN-1, 50 μ M), bradykinin (BK, 50 nM) or substance P (SP, 50 nM) into the coronary inflow tract.

Figure 3 Decrease in coronary perfusion pressure (CPP) (mmHg) by carbamoylcholine (CCh, 0.1 μ M), bradykinin (50 nM), substance P (50 nM), iloprost (0.1 μ M) and linsidomine (SIN-1, 5 μ M) in Langendorff hearts of control ($\square$) and hypercholesterolaemic rabbits ($\blacksquare$). The data are mean $\pm$ s.e.mean of $n = 8-9$ experiments. * $P < 0.05$ (cholesterol vs. control).

Figure 4 Release of NO (nm) and 6-oxo-PGF $_{1\alpha}$ (nm) by bradykinin (50 nM) and substance P (50 nM) into the coronary effluent of Langendorff hearts of control ($\square$) and hypercholesterolaemic rabbits ($\blacksquare$). The data are mean $\pm$ s.e.mean of the number of experiments indicated in the columns. * $P < 0.05$ (cholesterol vs. control).

Figure 5 Bradykinin-induced decrease in coronary perfusion pressure (CPP) (%) and release of NO (nm) in isolated hearts of control ($\square$) and hypercholesterolaemic rabbits ($\blacksquare$). Hearts were untreated (NT) or treated with superoxide dismutase (SOD, 10 u ml $^{-1}$). The data are mean $\pm$ s.e.mean of the number of experiments indicated in the columns. * $P < 0.05$ as indicated.

21 $\pm$ 6 mmHg in hearts of controls and by 18 $\pm$ 3 mmHg in hearts of hypercholesterolaemic rabbits. These differences were not significant ($P > 0.05$, $n = 8-9$).

L-NOARG at the same concentration markedly reduced bradykinin-induced NO formation in both groups to 0.7 $\pm$ 0.1 and 0.5 $\pm$ 0.1 nm, respectively. This was equivalent to an inhibition of stimulated NO release by 75–80% of control ($P < 0.05$). L-NOARG also reduced bradykinin-

Table 1 Effect of N G -nitro-L-arginine (L-NOARG, 30 μ M), L-arginine (L-Arg, 200 μ M) and superoxide dismutase (SOD, 10 u ml $^{-1}$) on bradykinin (50 nM)-stimulated 6-oxo-PGF $_{1\alpha}$ release (nm) in Langendorff hearts of control (Con) and cholesterol-fed rabbits (Chol)

Group	No treatment	+ L-NOARG	+ L-Arg	+ SOD
Con	0.44 $\pm$ 0.09	0.35 $\pm$ 0.08	0.30 $\pm$ 0.05	0.42 $\pm$ 0.11
Chol	0.99 $\pm$ 0.33	0.41 $\pm$ 0.11	0.36 $\pm$ 0.07	0.51 $\pm$ 0.15

The data are mean $\pm$ s.e.mean of $n = 4-9$ experiments; no significant differences between cholesterol and control groups ($P \geq 0.05$).

induced vessel relaxation in control hearts. In these preparations, bradykinin diminished the CPP by 21.0 $\pm$ 2.0% in the absence but only 13.0 $\pm$ 2.4% in the presence of L-NOARG ($P < 0.05$). In contrast, no inhibition of vessel relaxation by L-NOARG was obtained in hypercholesterolaemic hearts: the bradykinin-induced decrease in CPP was 9.3 $\pm$ 1.0% in the absence and 11.6 $\pm$ 1.5% in the presence of L-NOARG ($P > 0.05$, $n = 9$).

Effects of L-arginine (L-Arg)

Inhibition of bradykinin-induced NO formation by L-NOARG was partially restored by subsequent infusion of L-Arg (200 μ M) (cf. Figure 1). NO release was 1.65 $\pm$ 0.13 nm in controls and 1.98 $\pm$ 0.21 nm in hearts of hypercholesterolaemic animals. In controls, this was associated with a complete normalization of bradykinin-induced vasodilatation. No such effect was seen with L-Arg in the cholesterol group. There was still a significant reduction in endothelium-dependent relaxation ($P < 0.05$, $n = 8-9$). L-Arg did not alter the basal CPP (Figure 1).

Effects of superoxide dismutase (SOD)

In separate experiments, SOD (10 u ml $^{-1}$) was infused and, after a period of equilibration, bradykinin was administered as described above. No direct action of SOD on basal CPP, LVP or heart rate was observed at this concentration. In the presence of SOD, bradykinin-induced vasodilatation was significantly enhanced in both groups of hearts by comparable amounts. Thus, there was no specific effect of SOD on the CPP of normo- or hypercholesterolaemic hearts, respectively. Bradykinin-induced NO release and 6-oxo-PGF $_{1\alpha}$ formation were also not significantly altered by SOD treatment ($P > 0.05$, $n = 4-9$) (Table 1, Figure 5).

Discussion

This study demonstrates impaired endothelium-dependent relaxation in the coronary microcirculation of the rabbit at early stages of atherosclerosis, i.e. when overt plaque formation has not yet occurred. This confirms earlier data in coronary arteries and microvessels of cholesterol-fed rabbits (Osborne *et al.*, 1989), pigs (Cohen *et al.*, 1988) and monkeys (Sellke *et al.*, 1990) as well as several *in vivo* and *in vitro* studies on atherosclerotic human coronary arteries (Ludmer *et al.*, 1986; Bossaller *et al.*, 1987; Förstermann *et al.*, 1988). There was a reduced coronary vasodilatation against a variety of different endothelium-dependent relaxants, such as muscarinic agonists, bradykinin and substance P, suggesting that mechanisms distal to the receptor level are involved. Two possibilities exist: reduced receptor-dependent generation of vasodilator mediator(s) or reduced action of the mediator(s).

Reduced generation of endothelium-dependent vasodilators, such as NO or PGI $_2$, appears to be an attractive hypothesis

to explain reduced endothelium-dependent relaxation. However, in the present study, direct measurements of NO and PGI₂ clearly indicate that their formation is not reduced but rather enhanced in the coronary microcirculation. Beetens *et al.* (1986) described a biphasic change of vascular PGI₂ formation in cholesterol-fed rabbits. There was an enhanced 6-oxo-PGF_{1 α} production by aortic segments in early stages of cholesterol feeding which was followed by a severely depressed formation at later times. Voss *et al.* (1983) reported that the amount of PGI₂ generation depends on the type of vascular tissue studied. Thus, it appears to be clear from the data from the present study that any reduced endothelium-dependent relaxation cannot be explained by reduced PGI₂ production. There is also no evidence for reduced sensitivity of the coronary vessels to PGI₂ as seen from the unchanged responses to iloprost.

Our results also demonstrate that there was unchanged basal NO release but enhanced stimulated NO release into the vessel lumen of hypercholesterolaemic hearts. This finding agrees with a previous report by Minor and colleagues (1990) who, using a chemiluminescence technique, found enhanced release of nitrogen oxides, i.e. mainly nitrite, from aortae of hypercholesterolaemic rabbits. The oxyhaemoglobin technique used in the present study is more selective for NO, and does not detect NO metabolites (Woditsch & Schrör, 1992). In addition, the NO signal was markedly suppressed by L-NOARG, confirming both the specificity of the assay and the endogenous NO biosynthesis from L-Arg.

There are several possible sources for enhanced NO formation in hypercholesterolaemia. First, hypercholesterolaemia *per se* may result in changes of the abundance of vascular NO synthases. Distinct isoforms may exist (Moncada, 1992) and may be regulated in different ways (Lamas *et al.*, 1992). Recently, induction of a non-endothelial NO synthase has been postulated from bioassay experiments in the hypercholesterolaemic rabbit aorta (Verbeuren *et al.*, 1993). Cell culture studies suggest an upregulation of cytokine-induced NO synthase in cholesterol-enriched vascular smooth muscle cells (Pommerantz *et al.*, 1993). These data as well as those from Kanno and colleagues (1993) would probably explain our findings of increased stimulated NO release in terms of cytokine-induced expression of new NO synthases in hypercholesterolaemia.

The question still remains why enhanced expression of the enzyme and enhanced NO liberation still results in impaired endothelium-dependent relaxation. The theoretical possibility of exhaustion of substrate supply can be excluded as in this study and in two earlier *in vitro* trials (Mügge & Harrison, 1991; Kerr *et al.*, 1992) no improvement of the impaired endothelium-dependent vessel relaxation was observed by addition of L-Arg.

Diminished endothelium-dependent relaxation may result from a more rapid inactivation of released NO. There is evidence for enhanced endothelium-derived superoxide anion production in hypercholesterolaemia (Ohara *et al.*, 1993) and improved recovery of EDRF-dependent relaxation by superoxide dismutase (SOD) in a previous (Mügge *et al.*, 1991) and in the present study, SOD also significantly enhanced the bradykinin-induced relaxation in both hypercholesterolaemic and control hearts but did not normalize the reduced endothelium-dependent relaxation of hypercholesterolaemic hearts. This suggests that inactivation of NO by superoxide radicals may not fully explain impaired endothelium-dependent relaxation in this model. Other mechanisms, such as inactivation of NO by low-density lipoproteins (Jacobs *et al.*, 1990) may be involved.

Finally, the possibility exists that NO is less active on vascular smooth muscle because of disturbed signal transduction within the vascular smooth muscle cell. If this were true, then relaxation by exogenous NO donors should also be disturbed. In fact, relaxations subsequent to SIN-1 were significantly reduced in coronary arteries from hypercholesterolaemic animals. SIN-1 has previously been shown *in vitro* to release NO spontaneously and almost exclusively into the vessel lumen (Schrör *et al.*, 1991). This effect was not changed by SOD. Thus, decreased relaxation by SIN-1 may rather suggest a diminished sensitivity of the cholesterol-loaded vascular smooth muscle cell to cyclic GMP-related relaxation. This hypothesis is in agreement with studies by others (Verbeuren *et al.*, 1990; Tagawa *et al.*, 1991) and suggests a reduced action of NO within the vessel lumen on vessel tone in hypercholesterolaemia.

In summary, this study demonstrates an enhanced vascular NO forming capacity despite of impaired endothelium-dependent relaxation in the coronary microcirculation of hypercholesterolaemic rabbits. The decreased vasodilator activity of NO might be due to a more rapid local inactivation by superoxide radicals or oxidized LDL in the subendothelial space. Additionally, cholesterol-loaded, cytokine-exposed vascular smooth muscle cells may become less sensitive to the relaxing action of NO. There is no altered formation or reduced action of prostacyclin.

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