Oxidized LDL Increases the Sensitivity of the Contractile Apparatus in Isolated Resistance Arteries for Ca\textsuperscript{2+} via a Rho- and Rho Kinase–Dependent Mechanism

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**Background**—Oxidized LDL reduces NO-mediated and endothelium-derived hyperpolarizing factor–mediated dilations. We studied, in hamster skeletal muscle resistance arteries (213±8 μm; n=51), whether an altered vascular smooth muscle (VSM) response, particularly sensitization of the VSM contractile apparatus to Ca\textsuperscript{2+}, is involved in this oxLDL effect.

**Methods and Results**—VSM or endothelial [Ca\textsuperscript{2+}], and vascular diameter were measured in response to norepinephrine (0.3 μmol/L), sodium nitroprusside (10 μmol/L), C-type natriuretic peptide (1 to 100 nmol/L), papaverine (0.1 to 10 μmol/L), or the endothelial agonist acetylcholine (ACh, 0.01 to 1 μmol/L). OxLDL significantly increased resting VSM [Ca\textsuperscript{2+}], (11±3%), decreased diameter (8±2%), and enhanced norepinephrine-induced contractions. Dilations to sodium nitroprusside and C-type natriuretic peptide were significantly reduced (by 10±2% and 35±6%), whereas dose-response curves for papaverine and ACh were shifted to the right, despite unchanged increases in endothelial Ca\textsuperscript{2+} after ACh. OxLDL significantly shifted the Ca\textsuperscript{2+}-diameter relation to the left, as assessed by stepwise increasing extracellular Ca\textsuperscript{2+} (0 to 3 mmol/L) in depolarized skeletal muscle resistance arteries. This sensitization to Ca\textsuperscript{2+} by oxLDL was abolished after inhibition of Rho (C3 transferase) or Rho kinase (Y27632).

**Conclusions**—OxLDL reduces VSM responsiveness to vasodilators by increasing VSM Ca\textsuperscript{2+} but preferentially by sensitizing VSM to Ca\textsuperscript{2+} via a Rho- and Rho kinase–dependent pathway. *(Circulation. 2000;102:2402-2410.)*

**Key Words:** muscle, smooth ■ endothelium ■ endothelium-derived factors ■ atherosclerosis

Atherosclerosis and hypercholesterolemia are closely associated with changes of arterial vascular reactivity, such as attenuation of endothelium-dependent dilation and enhanced contractile responsiveness.\textsuperscript{1,2} In particular, nitric oxide (NO)–mediated dilation is impaired, as demonstrated in the human coronary circulation and in the human forearm.\textsuperscript{1,3} Oxidative modification of LDLs (oxLDL) to various degrees is considered to be a key event in the development of altered vascular reactivity.\textsuperscript{4,5} \textsuperscript{5} Experimental studies have provided evidence that oxLDL interferes with vascular reactivity at multiple levels: oxLDL reduces formation\textsuperscript{6} and stability of endothelium-derived NO,\textsuperscript{9,10} attenuates the activity of the cGMP-producing soluble guanylyl cyclase,\textsuperscript{11,12} and potentiates agonist-induced contractile responses.\textsuperscript{13,14} The majority of these studies have been performed in large conduit vessels, whereas comparatively little is known about the impact of oxLDL on vascular reactivity in the microcirculation. Microvascular responses are of particular interest because peripheral vascular resistance is increased in hypercholesterolemia and atherosclerosis,\textsuperscript{15,16} mainly as a result of increased vascular smooth muscle tone in microvessels. This increase in vascular smooth muscle tone can be caused by 2 major mechanisms: impairment of endothelium-dependent dilations and/or enhancement of the contractile response to agonists.

Regulation of endothelium-dependent dilation in the microcirculation differs from regulation in large vessels. In the microcirculation, the endothelium-derived hyperpolarizing factor (EDHF) plays a predominant role compared with NO. In contrast, in large vessels, NO appears to be the dominant factor. Because the impact of oxLDL on EDHF effects in microvessels has not been investigated, a goal of this study was to investigate the effect of oxLDL on EDHF-mediated dilation in skeletal muscle resistance arteries of the hamster. Because oxLDL could sensitize smooth muscle cells for calcium to cause vasoconstriction independent of endothelial influences, another goal was to study the effect of oxLDL on arteriolar constrictor responses in relation to smooth muscle calcium. Contractile responses are regulated not only by the cytosolic calcium concentration via Ca\textsuperscript{2+}-dependent myosin light chain phosphorylation but also by changes in the activity...
of the smooth muscle myosin phosphatase, which is regulated by a Rho-associated kinase.\(^4\) Thus, to investigate whether oxLDL acts primarily through an increase in \([Ca^{2+}]_i\), or an increase in calcium sensitivity, changes in \([Ca^{2+}]_i\), and diameter were determined simultaneously. The involvement of Rho and the Rho-associated kinase in oxLDL-dependent effects was studied further, with C3 transferase used as a specific inhibitor of Rho.\(^5\) To achieve a sufficient level of C3 transferase in the cytosol of vascular smooth muscle cells in intact resistance arteries, the arteries were incubated for 15 to 17 hours with a solution containing culture medium, antibiotics, the transfectant TransLT (20 \(\mu L/mL\)), and the C3 transferase protein (20 \(\mu g/mL\)), making use of a newly established vessel culture system described elsewhere in more detail.\(^6\) Possible unspecific effects of the transfection method were tested by comparing the vascular responses in nontreated and transfected vessels that were identical in both groups.

### Methods

**Drugs**

The MOPS-buffered salt solution was composed as follows (mmol/L): NaCl 145, KCl 4.7, CaCl\(_2\) 1.5, MgSO\(_4\) 1.17, NaH\(_2\)PO\(_4\) 1.2, pyruvate 2.0, EDTA 0.02, MOPS 3.0, and glucose 5.0. Fura 2-AM (Molecular Probes) was dissolved in water-free DMSO and stored as a 1 mmol/L stock solution (1 mg fura 2-AM in 1 mL DMSO). The solvent DMSO in the respective final concentrations had no effects on either vascular tone or sensitivity to vasoconstrictors or vasodilators. Norepinephrine (NE), acetylcholine (ACh), sodium nitroprusside (SNP), C-type natriuretic peptide (CNP), N\(^-\)nitro-l-arginine, and papaverine were purchased from Sigma Chemical Co; MnCl\(_2\), from Merck; and 5-nitrosod-N-acetyl-D,L-penicillamine (SNAP) from Alexicis Chemicals. Felodipine was a generous gift from Astra Chemicals (Wedel, Germany). C3 transferase was a generous gift from Dr Martin Aepfelbacher, Department of Microbiology, Ludwig Maximilians University, Munich, Germany. Y27632 ([6-(4-carboxyphenyl)-6,7-dihydro-1,2,4-triazolo[1,5-a]pyridazine-3-carboxamide] kindly provided by Yoshitomi Pharmaceutical Industries, Ltd, Osaka, Japan. The transfectant TransLT was purchased from PanVera Corp.

Concentrations given in the text refer to the final bath concentration.

**Isolation and Oxidation of LDL**

Human LDL was isolated and oxidized as described recently.\(^1\) Briefly, the native lipoprotein was prepared from pooled fresh human plasma. Antioxidant-free LDL (1 mg protein/mL) was incubated with CuSO\(_4\) (1 \(\mu mol/L\)) in PBS for 24 hours at 23°C. The degree of oxidation was quantified by the increase in relative mobility on agarose gel, indicating an enhanced negative charge of oxidized lipoprotein. Homogeneity of lipoproteins was tested by agarose gel electrophoresis (REP-HDL-Plus cholesterol electrophoresis; Helena Diagnostika). The mobility of oxLDL on agarose gel electrophoresis as an index for lipoprotein oxidation was 2.5- to 3.0-fold increased compared with native LDL. Protein content of oxLDL and native LDL was measured with a commercially available kit (Sigma protein kit), which is based on a modification of a method initially described by Lowry et al.\(^1\) LDL concentrations are always given as \(\mu g\) protein/mL solution. Lipoproteins were stored at 4°C in the dark and freshly prepared every 2 weeks. During this period, apolipoprotein B was intact and not degraded.

**Preparation of Small Resistance Arteries**

The care of the animals and the experimental procedures performed in this study were in strict accordance with the standards and guidelines provided by German animal protection laws. The preparations of the vessels and the technique of calcium and diameter measurements were previously described in more detail.\(^2\) Female golden Syrian hamsters were first anesthetized by intraperitoneal injection and then killed by intracardial injection of pentobarbital sodium (50 mg/kg). Segments of small resistance arteries were excised from the gracilis muscle and cannulated with glass micropipettes. The vessel and cannulation apparatus was mounted on the stage of a modified inverted microscope (Nikon, Diaphot 300) that was equipped with a video camera system. The transmural pressure for the arteriole was set hydrostatically to 45 mm Hg.

**Inhibition of Rho in Intact Small Resistance Arteries**

Rho was specifically inhibited by use of Clostridium botulinum exoenzyme C3 transferase, an ADP ribosyltransferase that acts on Rho. Its use is hampered by the difficulty of introducing the intact protein into cells.\(^3\) To achieve a sufficient level of C3 transferase in the cytosol of vascular smooth muscle cells in intact resistance arteries, the arteries were incubated for 15 to 17 hours with a solution containing culture medium, antibiotics, the transfectant TransLT (20 \(\mu L/mL\)), and the C3 transferase protein (20 \(\mu g/mL\)), making use of a newly established vessel culture system described elsewhere in more detail.\(^2\) Possible unspecific effects of the transfection method were tested by comparing the vascular responses in nontreated and transfected vessels that were identical in both groups.

**Measurements of [Ca\(^{2+}\)], and Outer Diameter**

The segments were incubated with MOPS-buffered saline containing 2 mmol/L fura 2-AM and 0.5% BSA. Dye loading of smooth muscle cells was terminated after 120 minutes by washing with MOPS saline. Selective loading of the endothelium in separate experiments was achieved by perfusion (1 mL/h) of the vessel with MOPS-buffered saline containing 2 mmol/L fura 2-AM and 0.5% BSA. After 60 minutes, the incubation buffer was exchanged for pure MOPS-buffered saline, which terminated the loading of endothelial cells. Fluorescence emitted by fura 2-AM at 510 nm after alternating excitation at 340 nm and 380 nm (PTI Deltascan, Photomet GmbH) was detected by means of a photomultiplier tube. The fluorescence ratio \(F_{340nm}/F_{380nm}\) was calculated after subtraction of the background fluorescence (obtained after fura 2-AM quenching with 8 mmol/L MnCl\(_2\)). The low concentration of fura 2-AM used in this study minimized any calcium-buffering effects of the fura 2, such that the responses to norepinephrine, angiotensin II, and step changes in transmural pressure were similar in loaded and unloaded arterioles. For measurements of vessel diameter, simultaneous videomicroscopy was performed at wavelengths >610 nm, which did not interfere with the fura 2-AM-related wavelengths.

**Experimental Protocols**

Experiments were started 30 minutes after termination of fura 2-AM loading. Changes of outer vascular diameter and fura 2-AM fluorescence were continuously recorded in 47 vessels isolated from 47 animals. All vessels that were studied developed 8.3 ± 2% spontaneous tone. The viability of each vessel was further assessed by its responsiveness to norepinephrine (NE, 0.1, 0.3, 1 \(\mu mol/L\)) as well as by its dilation after subsequent addition of acetylcholine (ACh, 1 \(\mu mol/L\)). All experiments were carried out in the presence of indomethacin (30 \(\mu mol/L\)) as 

\[\text{diameter}_{\text{vasodilator}} = \frac{\text{diameter}_{\text{baseline}} - \text{diameter}_{\text{vasodilator}}}{\text{diameter}_{\text{vasodilator}}} \times 100, \]  

with \(\text{diameter}_{\text{vasodilator}}\) representing the steady-state outer diameter after 2 minutes of stimulation.
with the respective vasodilator. Dia_{basal} is the steady-state outer diameter on stimulation with NE and dia_{max} the maximal diameter of the resistance arteries at a transmural pressure of 45 mm Hg. Because potential errors might prevent exact determination of [Ca^{2+}], in intact vessels, [Ca^{2+}]; was assessed as percent changes of emission ratio only. [Ca^{2+}]i in response to vasoconstricting agonists are expressed as Δ[Ca^{2+}]=[(R_{vaso}/R_{basal})×100]−100, whereas the changes in [Ca^{2+}]i in response to vasodilators are normalized to the ratio found in the preactivated state, [(R_{vaso}/R_{basal})×100]−100. According to calibration curves obtained in a cell-free system, the range of ratios observed here (0.4 to 36) fitted well into the linear range of the calibration curve (42.2 to 1520 nmol/L), which is a prerequisite for calculating accurate percent changes.

All results are presented as mean±SEM of n experiments, with n representing the number of vessels used per experimental series. Steady-state values of different experimental groups were compared by Student’s t test for paired data and when performing multiple comparisons, corrected according to Bonferroni. Differences were considered significant at a (corrected) error probability of P<0.05.

**Results**

**Incubation With OxLDL**

Incubation of the resistance arteries with 100 μg/mL oxLDL over a period of 10 minutes induced a significant rise in smooth muscle [Ca^{2+}];, by 11±3% (from R 3.53±0.33 to 3.92±0.37, P<0.05, n=24) associated with a decrease in resting diameter by 8±2% (from 240±6 to 220±7 μm, P<0.05, n=24; see Figure 1). The increase in [Ca^{2+}]; elicited by oxLDL was biphasic (Figure 1). The second phase (plateau) was abolished when oxLDL was applied in Ca^{2+}-free MOPS-buffered saline (n=6) and significantly reduced (by 92±4%, n=6) by blockade of L-type channels with felodipine (1 μmol/L; Figure 1). The concomitant increase in basal tone was almost completely abolished in calcium-free MOPS-buffered saline (1.3±0.7%) or with felodipine (1.7±0.6%).

**Effects of OxLDL on Endothelium-Independent Vasodilators**

NE induced a biphasic rise in smooth muscle [Ca^{2+}];, with a plateau at 45±3% (from 3.53±0.33 to 5.09±0.41, P<0.05, n=24) followed by a constriction of the vessel (by 44±1%, P<0.05, n=24). Steady-state [Ca^{2+}];, was 25±3% in the presence of oxLDL (increase from 3.92±0.37 to 4.72±0.37, P<0.05, n=24); the resulting constriction was augmented by 7±1% (P<0.05).

Subsequent application of the soluble guanylyl cyclase activator SNP (0.1, 1, and 10 μmol/L) elicited dilations by 40±9%, 73±8%, and 88±5%, respectively, which were significantly reduced to 11±3%, 54±8%, and 79±5% in the presence of oxLDL (P<0.05, n=6, Figure 2). As shown previously, dilations induced by NO, as derived from SNP or SNAP, are not associated with decreases in smooth muscle [Ca^{2+}];, in these vessels.

Dilations elicited by 1, 10, and 100 mmol/L CNP (by 14±7%, 55±9%, and 78±7%, n=6), which stimulates particulate guanylate cyclase, were also significantly reduced in the presence of oxLDL (to 9±5%, 30±9%, and 51±9%). Like SNP, CNP had no effect on smooth muscle [Ca^{2+}];, (data not shown).

The dose-response curve of papaverine-induced dilations in NE-preconstricted microvessels was shifted rightward by oxLDL (n=5, Figure 2). Papaverine-induced dilations were not associated with changes in Ca^{2+};.

**Effects of OxLDL on Endothelium-Dependent EDHF-Mediated Dilations**

The release of the endothelial autacoid EDHF by ACh has been shown to be calcium-dependent. The hyperpolarization caused by this factor results in decreased smooth muscle...
[Ca^{2+}], and vasodilation of the vessel. Incubation with 100 μg/mL oxLDL had no effect on endothelial resting [Ca^{2+}], or the ACh-induced increase in [Ca^{2+}], (n=4, Figure 3). Likewise, ACh (0.01, 0.1, and 1 μmol/L) caused concentration-dependent decreases in smooth muscle [Ca^{2+}], (0.3±1%, 29±7%, and 28±4%) that were not affected by oxLDL (2±3%, 29±7%, and 31±2%, n=6). However, corresponding ACh-induced dilations (16±5%, 64±8%, and 74±4%) were reduced in the presence of oxLDL (4±1%, 10±1% [P<0.05], and 60±7%, Figure 3).

Modulation of Smooth Muscle Calcium Sensitivity by OxLDL
The apparent calcium sensitivity of intact resistance arteries was assessed under depolarizing (120 mmol/L K+ in the external bath solution) conditions by stepwise increases in extracellular calcium from 0 to 3 mmol/L. Because depolarization opened the voltage-operated channels, these increases in extracellular Ca^{2+} were paralleled by increases in smooth muscle [Ca^{2+}]. As shown in the Table and Figure 4, despite similar increases in [Ca^{2+}] in the control and the oxLDL-

![Figure 2](image1)

**Figure 2.** OxLDL (100 μg/mL) uniformly reduced efficacy of a variety of endothelium-independent vasodilators (SNP, n=6; CNP, n=6; and papaverine [PAV], n=5) that elicit dilation via different cellular pathways. Values are mean±SEM. *Significant differences between groups.

![Figure 3](image2)

**Figure 3.** OxLDL (100 μg/mL) did not affect increases in endothelial [Ca^{2+}] (right, n=4) or decreases in smooth muscle [Ca^{2+}] (top left, n=6) as induced by ACh. However, despite normal decreases in smooth muscle [Ca^{2+}], dilations were significantly reduced in presence of oxLDL. ACh-induced effects on vascular smooth muscle were mediated by EDHF, because they occurred in presence of indomethacin and N^ω-nitro-L-arginine (30 μmol/L each). Values are mean±SEM. *Significant differences between groups.
treated groups, resulting constrictions were significantly augmented after treatment with oxLDL. This suggested an augmentation of the calcium sensitivity of the contractile apparatus induced by oxLDL. The apparent calcium-sensitizing effects of oxLDL were completely reversed 15 minutes after washout.

Effects of Inhibition of Rho and the Rho-Associated Kinase on oxLDL-Induced Ca2+-Sensitizing Effects

The involvement of Rho in oxLDL-induced effects was studied in vessels in which smooth muscle cells were transfected with the specific Rho inhibitor C3 transferase from *C botulinum*. To determine whether the Rho-activated Rho kinase downstream from the signaling pathway was involved in mediating the oxLDL effects, Rho kinase was blocked with the specific inhibitor Y-27632 in a concentration (1 μmol/L) that did not affect basal tone (198 ± 6 μm in control versus 195 ± 18 μm in Y-27632–treated vessels, n = 5) or NE-induced constrictions (109 ± 4 versus 115 ± 5 μm, n = 5). The decrease in basal diameter during incubation with oxLDL (−13 ± 2%) was completely inhibited by Y-27632 (2 ± 1%, P < 0.01, n = 6, Figure 5), whereas the oxLDL-induced increases in [Ca2+] were not affected by Rho-kinase inhibition (10 ± 4% versus 11 ± 3%, Figure 5).

Comparison of the calcium sensitivity under control conditions and after incubation with 100 μg/mL oxLDL, oxLDL + 20 μg/mL C3 transferase, or oxLDL + 1 μmol/L Y-27632 indicated that ADP ribosylation of Rho by C3 transferase and Rho-kinase inhibition by Y-27632 completely reversed the calcium-sensitizing effect of oxLDL (Figure 6). Inhibition of Rho or Rho kinase had no effect on the rises in [Ca2+] after stepwise increase of extracellular Ca2+ in depolarized vessels.

Discussion

This study demonstrates an enhanced contractile activation of microvascular smooth muscle by oxLDL, resulting in an increased vascular tone and a reduced response to vasodilators. Although oxLDL moderately increases smooth muscle calcium, a calcium-sensitizing mechanism seems to be functionally more important for this effect. We suggest that the

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**Figure 4.** Assessment of apparent calcium sensitivity of resistance arteries by stepwise increases in extracellular Ca2+ (0 to 3 mmol/L) under depolarizing conditions (120 mmol/L K+ in organ bath). As Ca2+ was increased in organ bath, resulting constrictions were significantly enhanced in presence of oxLDL (left tracing). Right, Graph shows steady-state diameters after 2 minutes plotted over respective [Ca2+] level (n = 7, expressed as ratio F340nm/F380nm). Values are mean ± SEM. *Significant differences between diameters in absence vs diameters in presence of oxLDL.
sensitization to calcium is mediated by a Rho and Rho kinase–dependent pathway that most likely targets regulation of myosin light chain phosphatase activity.29–32

The increase in basal microvascular tone as well as the enhanced response to norepinephrine as an acute effect of oxLDL is in agreement with previous studies performed in large conduit arteries.13 In our study, the state of enhanced smooth muscle contractile activation had the further effect of uniformly reducing the efficacy of the various vasodilators. This impaired vasodilation was not mechanistically selective, because it was equally pronounced for the cGMP-mediated vasodilators NO and CNP, for papaverine, and for EDHF. Because endothelium-dependent as well as endothelium-independent dilations were affected, the main site of the inhibiting action of oxLDL appears to be at the smooth muscle level. This is further supported by the observation that oxLDL, in the short term, did not impair muscarinic receptor function or receptor coupling, because the calcium response to ACh in endothelial cells was not affected. We have shown before that on stimulation with ACh, the endothelium of these vessels produces an EDHF that activates K_{Ca} channels and decreases smooth muscle calcium, thereby inducing vasodilation.24,27 OxLDL did not affect this EDHF-induced calcium decrease in the vascular smooth muscle, which suggests that the synthesis and stability of EDHF was not altered by oxLDL. Nevertheless, EDHF-induced dilation was significantly reduced. Of note, the EDHF-induced dilation was reduced even though the decrease of intracellular free calcium was identical with the control situation. This points toward an alteration of the calcium sensitivity of the smooth muscle contractile apparatus by oxLDL.

An increase in calcium sensitivity was directly implicated by comparing the degree of vasoconstriction after stepwise increases in calcium in the absence or presence of oxLDL. At any given calcium concentration in vascular smooth muscle cells, there was a significantly higher level of constriction after treatment with oxLDL. In a limited series of experiments, this Ca^{2+}-sensitizing effect could also be demonstrated in endothelium-denuded vessels (n=4, data not shown).

There is growing evidence that the modulation of calcium sensitivity of vascular smooth muscle is an important mechanism for the regulation of vascular tone.33,34 Indeed, we have shown previously that cGMP-mediated dilation occurred without a concomitant decrease in [Ca^{2+}], in hamster resistance arteries.24 It was recently demonstrated that cGMP, in addition to stimulation of Ca^{2+}-ATPases, activates myosin light chain phosphatase, thereby decreasing myosin light chain phosphorylation and hence the apparent sensitivity of the contractile apparatus to calcium.34 Conversely, a reduced myosin light chain phosphatase activity results in an increased smooth muscle tone.17 The regulation of the MLC-phosphatase is not yet completely understood. However,
recent data have identified myosin light chain phosphatase as a target of Rho kinase that is regulated by the small GTP-binding protein, RhoA.31 Furthermore, another recent study demonstrated that the Rho/Rho-kinase pathway could be activated by mildly oxidized LDL in endothelial cells.35 Because in our experiments, specific inhibitors of Rho and Rho-associated kinase, C3 transferase and Y27632,20 completely antagonized the calcium-sensitizing effect of oxLDL, we propose that the sensitizing effect of oxLDL also results from an activation of the Rho–Rho-kinase pathway. Inhibition of Rho kinase did not affect Ca²⁺ entry mechanisms, because treatment with Y27632 abolished the oxLDL-induced constriction but did not affect the concomitant increase in [Ca²⁺]. The ability of C3 transferase and Y27632 to reduce vascular tone compared with control vessels suggests that selective manipulation of the Rho–Rho-kinase pathway is sufficient to modulate vascular tone. The effects were most pronounced at low and intermediate concentrations of smooth muscle calcium, indicating its potential relevance to the control of resting tone. Moreover, the experiments depicted in Figure 6 imply that this pathway has a role in determining vascular tone after initiation of constriction by an increase in Ca²⁺. For example, the initial constriction after readdition of 0.5 mmol/L external Ca²⁺ was similar in control and C3 transferase– or Y27632-treated segments; however, the steady-state constriction was attenuated in the latter. This is consistent with the idea that in C3 transferase– and Y27632-treated segments, an increased MLC-phosphatase activity opposes Ca²⁺-dependent activation of MLC kinase.

The introduction of C3 transferase to the cytosol of vascular smooth muscle cells in intact resistance arteries was achieved by use of a newly developed technique. Isolated vessels were kept in culture for 15 to 17 hours, and the smooth muscle cells on the abluminal side were exposed to culture medium containing the transfectant TransLT26 and the C3 transferase protein. The functional data obtained after transfection support the conclusion that an amount of protein sufficient to inhibit the GTPase Rho entered the smooth muscle cells. In general, the study of smooth muscle function is hampered by the fact that many specific inhibitors of key enzymes in signaling pathways leading to constriction are peptides or proteins that cannot be used in intact but only in skinned preparations. The new technique introduced here for the first time could help to overcome this limitation and allow studies in intact-vessel preparations.

It has been suggested that the increased contractile responsiveness in the presence of oxLDL is due to an elevated smooth muscle calcium. Calcium-increasing effects of oxLDL in smooth muscle cells have been demonstrated before in cell cultures.36 This effect is not restricted to smooth muscle cells; it has also been observed in cultured endothelial cells.37 The increase in [Ca²⁺] is apparently a prerequisite for
oxLDL-induced contractions, because they were abolished by calcium entry blockers. In accordance with these earlier studies, there was indeed an increased resting calcium level in smooth muscle cells of the small vessels studied here, which was abolished by the calcium entry blocker felodipine. This compound did not affect the transient initial increase in [Ca\(^{2+}\)], however, suggesting that the latter resulted from a release from intracellular stores. Nevertheless, felodipine completely blocked the oxLDL-induced constriction, indicating that sustained elevation of intracellular free calcium is a necessary step in activating the signaling cascade, leading to an increased calcium sensitivity that composes the Rho-kinase pathway. At present, it is not clear how oxLDL activates this pathway in microvascular smooth muscle cells. It has been shown, however, that in endothelial cells a decrease in [Ca\(^{2+}\)] is mandatory for an activation of RhoA, which in turn activates Rho kinase.\(^{38}\)

**Clinical Outlook**

It is widely thought that the association between hypercholesterolemia and arterial hypertension is mainly due to an oxLDL-induced endothelial dysfunction. Numerous studies focusing on endothelium-dependent dilation in hypercholesterolemia and atherosclerosis have indeed demonstrated that endothelium-dependent dilation mediated by NO is impaired. However, as demonstrated in our study, short-term exposure to oxLDL does not selectively impair endothelial function or the signaling cascade leading to EDHF release. In addition, the present study clearly shows that oxLDL, independent of any potential effects on endothelial function, increases the Ca\(^{2+}\) sensitivity of myofilaments in arteriolar vascular smooth muscle, possibly through stimulation of the small GTPase Rho and the Rho-associated kinase, resulting in enhanced vasoconstriction. Thus, this observation provides a new mechanistic explanation for increased peripheral resistance and hypertension as observed in hypercholesteremic patients. A previous study demonstrated that the use of Y-27632 reduced blood pressure in different models of hypertension. A previous study demonstrated that the use of Y-27632 might be therapeutically useful.\(^{20}\)

**Acknowledgments**

This work was supported by the Deutsche Forschungsgemeinschaft (SFB 553/B2). The authors would like to thank Prof Gerald A. Meininger, Texas A&M University Health Science Center (College Station), for many thoughtful discussions and constructive criticism in preparing the manuscript. The study contains data from the doctoral thesis of Roland Derwand.

**References**

27. Bolz SS, Fisslthaler B, Pieperhoff S, et al. Antisense oligonucleotides against cytochrome P450 2C8 attenuate EDHF-mediated Ca\(^{2+}\) changes


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Circulation. 2000;102:2402-2410
doi: 10.1161/01.CIR.102.19.2402

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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