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LDL Cholesterol Upregulates Synthesis of Asymmetrical Dimethylarginine in Human Endothelial Cells

Involvement of *S*-Adenosylmethionine–Dependent Methyltransferases

Rainer H. Böger, Karsten Sydow, Jürgen Borlak, Thomas Thum, Henrike Lenzen, Bibiana Schubert, Dimitrios Tsikas, Stefanie M. Bode-Böger

Abstract—Asymmetrical dimethylarginine (ADMA) is an endogenous nitric oxide synthase inhibitor. It is formed by protein arginine *N*-methyltransferases (PRMTs), which utilize *S*-adenosylmethionine as methyl group donor. ADMA plasma concentration is elevated in hypercholesterolemia, leading to endothelial dysfunction and producing proatherogenic changes of endothelial cell function. Four different isoforms of human PRMTs have been identified. Because the release of ADMA from human endothelial cells is increased in the presence of native or oxidized LDL cholesterol, we investigated the potential involvement of PRMT activity and gene expression in this effect. We found that the production of ADMA by human endothelial cells is upregulated in the presence of methionine or homocysteine and inhibited by either of the methyltransferase inhibitors *S*-adenosylhomocysteine, adenosine dialdehyde, or cycloleucine. This effect is specific for ADMA but not symmetrical dimethylarginine. The upregulation of ADMA release by native and oxidized LDL is abolished by *S*-adenosylhomocysteine and by the antioxidant pyrrollidine dithiocarbamate. Furthermore, a methyl-^{14}C label is transferred from *S*-adenosylmethionine to ADMA but not symmetrical dimethylarginine, in human endothelial cells. The expression of PRMTs is upregulated in the presence of native or oxidized LDL. Our data suggest that the production of ADMA by human endothelial cells is regulated by *S*-adenosylmethionine–dependent methyltransferases. This activity is upregulated by LDL cholesterol, which may be due in part to the enhanced gene expression of PRMTs. In concentrations reached by stimulation of methyltransferases (5 to 50 μmol/L), ADMA significantly inhibited the formation of ^{15}N-nitrite from L-[*guanidino*-$^{15}N_2$]arginine. These findings suggest a novel mechanism by which ADMA concentration is elevated in hypercholesterolemia, leading to endothelial dysfunction and atherosclerosis. **(*Circ Res*. 2000;87:99-105.)**

Key Words: nitric oxide synthase ■ dimethylarginine ■ lipoproteins ■ cholesterol ■ endothelium

Nitric oxide (NO) formed by functionally intact endothelial cells is an important mediator of vasodilation, a regulator of the interaction of platelets and leukocytes with the vascular wall, and an inhibitor of smooth muscle cell growth. Via these mechanisms, NO acts as an endogenous antiatherogenic molecule.[1] All known risk factors for atherosclerosis, like hypercholesterolemia, smoking, hypertension, and diabetes mellitus, are associated with reduced NO-dependent vasodilation, even before the development of clinically or morphologically apparent atherosclerosis.[1] One of the mechanisms that lead to this endothelial dysfunction is the accumulation of an endogenous inhibitor of NO synthase, asymmetrical dimethylarginine (ADMA).[2] Plasma levels of ADMA are elevated in hypercholesterolemic rabbits[3,4] and in patients with occlusive vascular disease[5] or hypercholesterolemia.[2] The elevation of ADMA is associated with reduced NO production and impaired endothelium-dependent vasodilation.[2–5]

The biosynthesis and metabolism of dimethylarginines are not completely understood. Endothelial cells are capable of synthesizing ADMA and, in minor amounts, symmetrical dimethylarginine (SDMA).[6,7] Dimethylarginines are probably formed from the degradation of methylated proteins.[8] In mammals, 2 distinct protein arginine methyltransferase activities have been identified.[9] One monomethylates and asymmetrically dimethylates arginine residues within the human heterogeneous nuclear ribonucleoprotein (hnRNP) A1 protein and other proteins (protein arginine *N*-methyltransferase type I),[10] whereas the other monomethylates and symmetrically dimethylates myelin basic protein in brain (protein arginine *N*-methyltransferase type II).[9] Thus, the non–myelin basic protein–specific isoform (type I enzyme activity) is the major source of asymmetrical methylarginines (ADMA and N^G-monomethyl-L-arginine), and these are the methylarginines that inhibit NO synthase activity. A major source of methyl

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groups used for various methylating reactions is *S*-adenosylmethionine, which is an intermediate in the conversion of methionine to homocyst(e)ine.[11,12]

Recently, 4 different isoforms of protein-arginine methyltransferases were characterized at the protein and genomic levels in human tissues or cells. They all share methyltransferase type I activity, but they differ in oligomerization, subcellular localization, substrate specificity, and regulation (for a review, see Gary and Clarke[13]). Human protein-arginine *N*-methyltransferase-1 (PRMT1) was identified as a protein that interacts with the intracellular domain of the interferon-α,β receptor, and its cDNA has been cloned and sequenced.[14,15] Moreover, database searches have identified a distinct human gene, HRMT1L1 (PRMT2 in the GenBank database).[16] PRMT3 was identified as another enzyme of this family with distinct enzymatic properties.[17] Finally, HRMT1L2 was detected in a yeast 2-hybrid screen.[18]

We hypothesized that the expression of protein arginine *N*-methyltransferases may be modulated in the presence of native and oxidized LDL cholesterol (nLDL and oxLDL, respectively), thus providing an explanation for an elevated ADMA concentration in hypercholesterolemia.[2,5] Therefore, the present experiment was designed to determine whether the formation of ADMA and SDMA by cultured human endothelial cells is regulated by the activity of *S*-adenosylmethionine–dependent protein arginine *N*-methyltransferases. Furthermore, we investigated whether the gene expression of 1 of the isoforms of protein-arginine *N*-methyltransferases is upregulated in the presence of nLDL and oxLDL cholesterol in cultured human endothelial cells.

Materials and Methods

Experimental Setting

ECV304 endothelial cells and primary human coronary artery endothelial cells (HCAECs) were cultured until subconfluence. Fresh medium with or without lipoproteins or the appropriate chemicals was added, and cells were incubated for 24 hours before the cells and incubation media were harvested for the measurement of ADMA and SDMA levels. nLDL was isolated from human plasma through sequential gradient ultracentrifugation as described by Pritchard et al.[19] oxLDL was prepared after the isolation of LDL by incubation with 5 μmol/L $CuSO_4$ (24 hours at 4°C).

For subsequent PCR analysis, endothelial cells were incubated for 24 hours in medium with or without nLDL or oxLDL. After incubation, medium was collected for the analysis of ADMA and SDMA, and cells were carefully detached with Accutase and rapidly frozen in liquid nitrogen for the isolation of total mRNA.

For experiments in which the incorporation of [^{14}C]methyl groups into dimethylarginines was studied, subconfluent cells were incubated in methionine-free medium in the presence of 50 μmol/L [^{14}C]-CH_3-*S*-adenosyl-methionine (2.7 TBq/mmol; Amersham Pharmacia) for 48 hours. After incubation, conditioned media were collected for HPLC analysis and liquid scintillation counting.

Analytical Methods

The concentrations of dimethylarginines were determined with HPLC according to a previously described method.[3] Homocyst(e)ine concentrations were measured with a commercially available fluorescence polarization assay (Abbott IMX; Abbott Diagnostics).[20] Endothelial cell NO synthase activity was determined by assessing the conversion of L-[*guanidino*-$^{15}N_2$]arginine to ^{15}N-nitrite with gas chromatography/mass spectrometry, according to a previously described method.[21]

RT-PCR

Total RNA was isolated from endothelial cells with the SV Total RNA Isolation System (Promega) according to the manufacturer's recommendations. Total RNA (4 μg) from each sample were used for RT. For PCR amplification of cDNA, a 25-μL reaction mixture was prepared that contained 10× polymerase reaction buffer (Roche), 3 mmol/L $MgCl_2$ (Roche), 0.4 mmol/L concentration of dNTPs (Roche), 200 nmol/L concentration of the 3′ and 5′ specific primers (GIBCO), 1 U *Taq*-polymerase (Roche), and 1 μL of cDNA. Amplified cDNA products were separated on a 1.8% agarose gel. Gels were stained with ethidium bromide and photographed on a transilluminator. Densities were quantified with NIH Image analyzer software. cDNA products were sequenced by Seqlab.

Chemicals

ADMA and SDMA were purchased from Alexis. We used the protein arginine *N*-methyltransferase inhibitors *S*-adenosylhomocysteine,[22] cycloleucine,[23] and adenosine-2,3-dialdehyde[24] (Sigma Chemie). Unless noted otherwise, all other drugs were also obtained from Sigma Chemie and were of the highest purity available.

Calculations and Statistical Analysis

All data are given as mean±SEM. Statistical significance was tested with ANOVA followed by Fisher's protected least significant difference test. Statistical significance was assumed for $P<0.05$.

An expanded Materials and Methods section is available online at http://www.circresaha.org.

Results

Effect of LDL Cholesterol on ADMA Release by Human Endothelial Cells

After an incubation period of 24 hours, baseline concentrations of ADMA and SDMA in ECV304 supernatants were 18.4±3.4 and 14.1±2.7 pmol/μg protein, respectively. In the presence of nLDL, there was a concentration-dependent increase in ADMA release (100 mg/dL nLDL, 22.2±2.4 pmol/μg protein; 300 mg/dL nLDL, 33.2±2.4 pmol/μg protein; $P<0.05$ versus control), whereas for SDMA there was only a slight, insignificant trend toward higher concentrations. The increase in ADMA release induced by 300 mg/dL LDL was reversed in the presence of the *N*-methyltransferase inhibitor *S*-adenosylhomocysteine (10 μmol/L, 9.3±0.5 pmol/μg protein; $P<0.05$ versus nLDL alone).

HCAECs released 15.1±2.9 pmol ADMA/μg protein and 8.0±1.7 pmol SDMA/μg protein under baseline conditions. nLDL and oxLDL induced concentration-dependent increases in ADMA release (Figure 1). This increase in ADMA production was significantly inhibited by the intracellular antioxidant pyrrollidine dithiocarbamate (PDTC; 1 μmol/L). SDMA levels were not significantly changed (data not shown).

Role of *N*-Methyltransferase Activity in ADMA Formation by Human Endothelial Cells

The baseline formation of ADMA by ECV endothelial cells was significantly inhibited by the *N*-methyltransferase inhibitors adenosine-2,3-dialdehyde (10 μmol/L), *S*-adenosylhomocysteine (10 μmol/L), and cycloleucine (10 mmol/L) by 44±5%, 62±8%, and 45±4%, respectively (each $P<0.05$ versus control; Figure 2). In HCAECs, the inhibition of ADMA release was 53±13%, 41±3%, and 37±7%, respectively (each $P<0.05$ versus control;

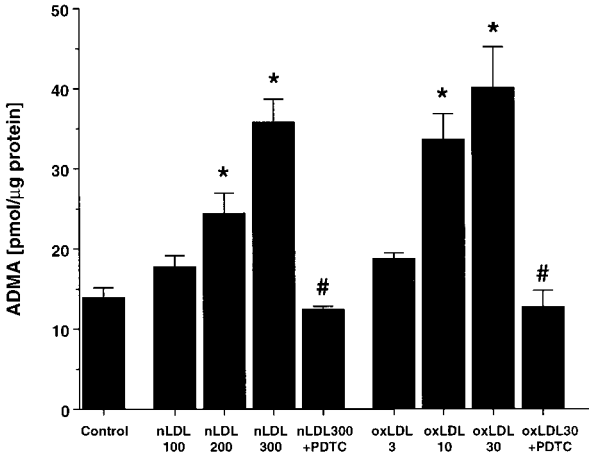

Figure 1. Concentration-dependent stimulation of ADMA release from primary HCAECs by nLDL (100 to 300 mg/dL) and oxLDL (3 to 30 mg/dL). Stimulation was reversed by the intracellular antioxidant PDTC (1 μmol/L). Data are mean±SEM of 4 to 6 experiments. *$P<0.05$ vs control. #$P<0.05$ vs without PDTC.

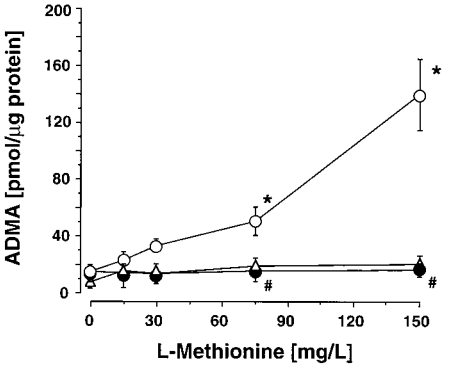

Figure 3. Effect of incubation of HCAECs in the presence of increasing concentrations of L-methionine on the formation of ADMA (○, ●) and SDMA (△). Note that the effect of methionine is relatively selective for ADMA. It is completely reversed in the presence of the methyltransferase inhibitor adenosine-2,3-dialdehyde (10 μmol/L; ●) compared with control conditions (○). Data are mean±SEM of 4 to 6 experiments. *$P<0.05$ vs control. #$P<0.05$ vs methionine alone.

Figure 2). SDMA release remained unchanged in the presence of these inhibitors in both types of endothelial cells (data not shown). Incubation of endothelial cells with higher concentrations of these methylation inhibitors induced cell death.

The formation of ADMA by ECV304 and by HCAECs was increased in the presence of L-methionine in a concentration-dependent manner (Figure 3). L-Methionine significantly increased homocysteine concentrations in ECV304 endothelial cell supernatants from 14.3±1.1 to 40.1±3.0 pmol/μg protein (4 to 7 μmol/L), indicating demethylation of L-methionine. Coincubation with adenosine-2,3-dialdehyde (10 μmol/L) completely abolished the increase in ADMA formation in the presence of L-methionine in both cell types (Figure 3). The incubation of HCAECs with DL-homocysteine also induced a significant increase in ADMA formation. The effect of homocysteine was further enhanced in the presence of vitamins B6 and B12 and folic acid (Figure 4). SDMA release was not significantly affected by L-methionine or DL-homocysteine (data not shown).

Origin of Methyl Groups Incorporated in ADMA and SDMA

When ECV304 endothelial cells were incubated in the presence of [^{14}C]-CH_3-*S*-adenosyl-methionine for 48 hours, a significant accumulation of radioactivity was observed in the HPLC fraction that corresponded to ADMA, whereas no radioactivity could be detected in the fraction that corresponded to SDMA (Figure 5). The accumulation of radiolabeled ADMA after incubation with [^{14}C]-CH_3-*S*-adenosyl-methionine was increased by ≈3-fold in the presence of nLDL cholesterol (417.6±96.5 versus 136.3±33.3 fmol · 48 h^{-1} · μg protein^{-1}; $P<0.05$).

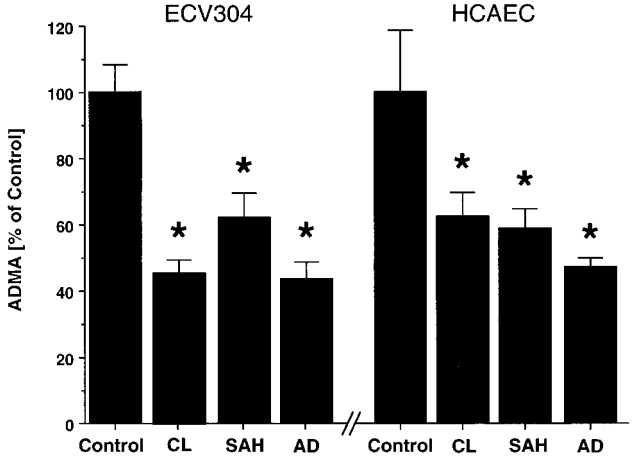

Figure 2. Effect of the inhibitors of *S*-adenosylmethionine–dependent methyltransferases cycloleucine (CL), *S*-adenosylhomocysteine (SAH), and adenosine-2,3-dialdehyde (AD; all 10 μmol/L) on accumulation of ADMA in conditioned media of human endothelial cells. Data are mean±SEM of 4 to 6 experiments. *$P<0.05$ vs control.

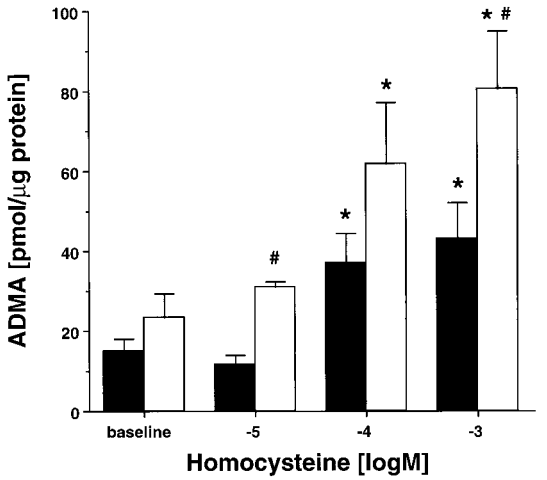

Figure 4. Effect of DL-homocysteine on ADMA release by HCAECs in the presence (open columns) or absence (filled columns) of folic acid (10 μmol/L), vitamin B6 (100 mmol/L), and vitamin B12 (10 mmol/L). Data are mean±SEM of 4 to 6 experiments. *$P<0.05$ vs control. #$P<0.05$ vs homocysteine alone.

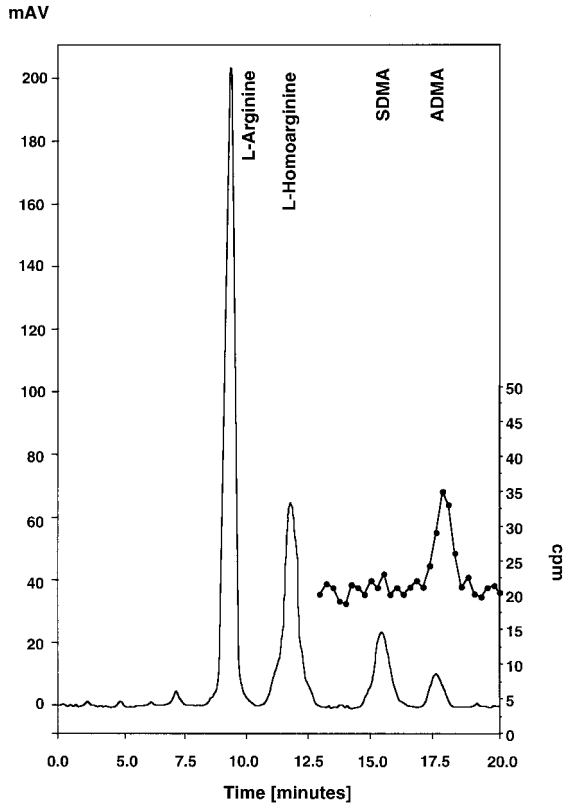

Figure 5. HPLC of the analysis of an endothelial cell supernatant after incubation with [^{14}C]-CH_3-*S*-adenosylmethionine. Radioactivity coeluted with the peak corresponding to the retention time of ADMA.

Effect of ADMA on Endothelial Cell NO Synthase Activity

ADMA induced a concentration-dependent inhibition of the formation of ^{15}N-nitrite from L-[*guanidino*-$^{15}N_2$]arginine (Figure 6). Significant inhibition was induced by ADMA concentrations of ≥5 μmol/L (24±7 pmol/μg protein).

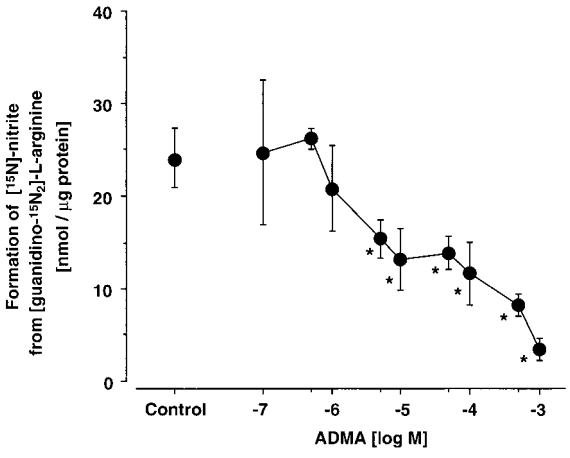

Figure 6. Effect of ADMA on NO elaboration by primary HCAECs. NO synthase activity was determined as the rate of conversion of L-[*guanidino*-$^{15}N_2$]arginine into ^{15}N-nitrite. Cells were preincubated with isotope-labeled arginine for 24 hours and stimulated with ATP (100 μmol/L) for 4 hours. Data are mean±SEM of 4 to 6 experiments for each point. *$P<0.05$ vs control.

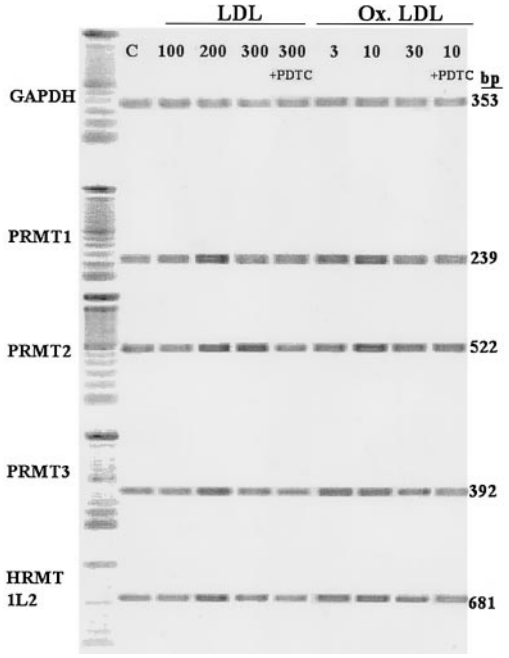

Figure 7. Ethidium bromide–stained agarose gel of a typical RT-PCR with primers specific for the 4 allelic variants of protein arginine *N*-methyltransferases in comparison with GAPDH. Total mRNA was isolated from HCAECs incubated under control conditions (controls) or under the conditions detailed in the legend.

Expression of Protein Arginine *N*-Methyltransferases

The expression of all 4 allelic variants of protein arginine *N*-methyltransferases (PRMT1, PRMT2, PRMT3, and HRMT1L2) was detected in nonstimulated ECV304 endothelial cells and in HCAECs. The effects of nLDL or oxLDL on protein arginine *N*-methyltransferase expression in HCAECs are shown in Figures 7 and 8. In HCAECs, both nLDL and oxLDL induced concentration-dependent increases in methyltransferase expression within a pathophysiologically relevant concentration range. The upregulation of mRNA expression was 1.5- to 2.5-fold in the presence of 200 or 300 mg/dL nLDL or 10 or 30 mg/dL oxLDL. The effects of nLDL and oxLDL on methyltransferase expression were reversed with PDTC. In ECV304 cells, PRMT1 expression was increased by ≈2-fold (range 1.4- to 2.2-fold), and PRMT2 expression was increased by 2- to 4-fold (range 1.3- to 5.6-fold) in the presence of nLDL (data not shown). The sequencing of RT-PCR products showed conformity of base sequences with the published sequence for each isoform (data not shown).

Discussion

The major findings of our study are that (1) *S*-adenosylmethionine is a source of methyl groups incorporated into ADMA by human endothelial cells, (2) the inhibition of *S*-adenosylmethionine–dependent methyltransferases results in reduced endothelial cell formation of ADMA, (3) 4 different protein arginine *N*-methyltransferases are expressed in human endothelial cells under baseline conditions, and (4) the gene expression of protein arginine *N*-methyltransferases is upregulated in the presence of nLDL or oxLDL

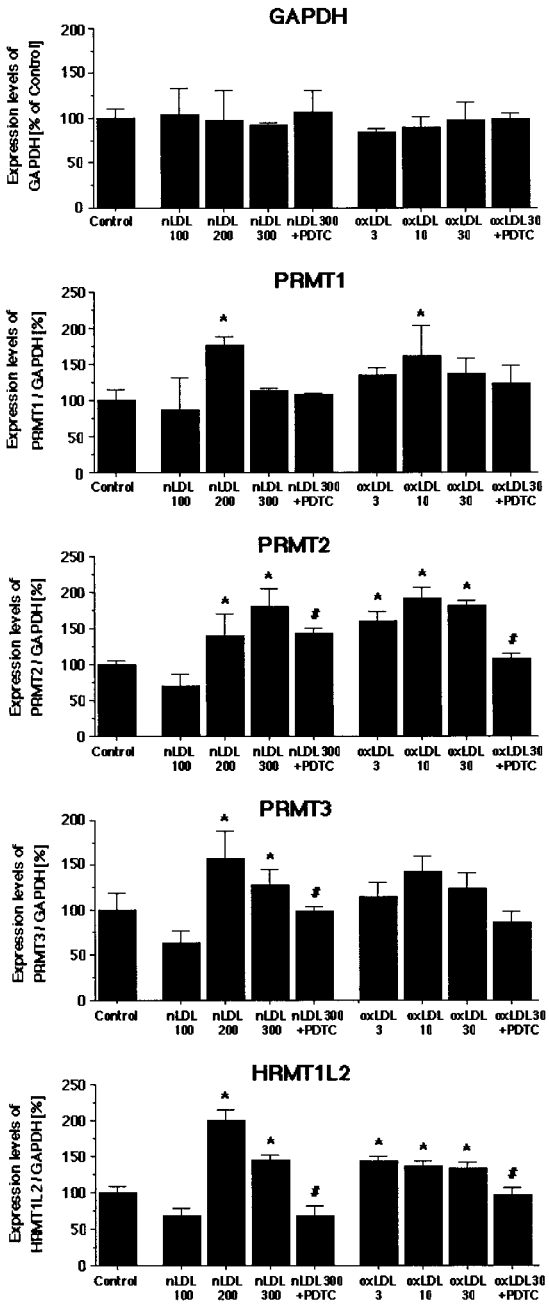

Figure 8. Expression levels of protein arginine *N*-methyltransferase isoenzymes in HCAECs. Data are mean±SEM of densitometric analysis of 4 to 6 independent experiments. *$P<0.05$ vs control.

cholesterol. This effect is mirrored in increased methyltransferase activity.

Several lines of evidence support our conclusion that *S*-adenosylmethionine is a methyl group donor in the synthesis of ADMA: First, the incubation of endothelial cells with L-methionine increased homocysteine concentration in conditioned media, suggesting that demethylation of methionine occurred. Simultaneously, there was a significant and concentration-dependent elevation of ADMA levels in the conditioned media. Second, homocysteine at concentrations considerably higher than those reached during the incubation with methionine also stimulated endothelial ADMA release but to a much lesser extent than methionine. This finding is in agreement with the hypothesis that *S*-adenosylmethionine is the methyl group donor for ADMA synthesis, because homocysteine must be remethylated before being able to contribute to ADMA synthesis and therefore would be expected to have a lesser influence on this pathway. Another explanation might be that homocysteine may be converted to *S*-adenosylhomocysteine, which inhibits methyltransferase-catalyzed reactions. In the present study, we did not measure *S*-adenosylhomocysteine levels. However, the finding that the addition of B vitamins enhanced the stimulatory effect of homocysteine on ADMA shows that increased turnover rate of the homocysteine–methionine methylation cycle also increases the formation of ADMA. Third, incubation with one of the inhibitors of protein arginine *N*-methyltransferase activity (*S*-adenosylhomocysteine, adenosine dialdehyde, or cycloleucine[22–24]) significantly reduced basal ADMA release by endothelial cells. Finally, the strongest evidence in favor of our hypothesis comes from our observation that after incubation with [^{14}C]-CH_3-*S*-adenosylmethionine, the isotope was detected in the HPLC fraction that coeluted with ADMA. A similar approach has previously led to the identification and biochemical characterization of protein arginine *N*-methyltransferase from yeast[12] and from various mammalian tissues.[10,24]

ADMA acts as an endogenous inhibitor of NO synthase in macrophages[25] and in endothelial cells.[7] In vitro, ADMA concentrations between 1 and 10 μmol/L reduce the activity of NO synthase in rat mesentery tissue[26] and in rat cerebral blood vessels.[27] The present data show that ADMA inhibits the conversion of L-[*guanidino*-$^{15}N_2$]arginine to ^{15}N-nitrite (a specific index of NO synthase activity[21]) in concentrations of $\geq$5 μmol/L (ie, levels also obtained through the stimulation of methyltransferases with methionine or by LDL cholesterol in the same cells). The inhibition of NO synthase by ADMA increases endothelial oxidative stress and upregulates the expression of redox-sensitive genes that encode for endothelial adhesion molecules,[7] comparable to that observed in early atherogenesis.[28] ADMA may therefore act as an endogenous proatherogenic molecule. In humans, hypercholesterolemia,[2] peripheral vascular disease,[5] and hypertension[29] are associated with elevated ADMA levels. In hypercholesterolemic humans, an elevated ADMA concentration is related to impaired endothelium-dependent vasodilation, a feature that is indicative of increased cardiovascular risk.[2]

We have previously reported that endothelial cells cultured in the presence of nLDL or oxLDL cholesterol release more ADMA into conditioned media than under control conditions.[7] This finding was recently confirmed by Ito et al.[30] Our present data further extend these observations by showing that the stimulatory effect of nLDL on ADMA release is completely abrogated in the presence of the methyltransferase inhibitor *S*-adenosylhomocysteine. Therefore, the modulation of methyltransferase activity may be involved in the stimulation of endothelial cell ADMA release by nLDL or oxLDL. Biochemical data suggest that although protein side chain arginine guanidino groups are known to serve as substrates for protein arginine *N*-methyltransferases, the release of free ADMA probably occurs during normal protein turnover. No

distinct demethylase has been identified for asymmetrically dimethylated arginine residues, although one has been suggested for myelin basic protein symmetrically dimethylated arginine.[31] Experiments with in vivo labeled histones from rat tissues also indicated that the turnover of the methylated arginine residues coincided with the degradation of the histones.[13,32]

Protein arginine *N*-methylation has been recognized in a number of eukaryotic proteins, but its functional significance is just beginning to be understood. Postulated roles for these posttranslational modifications include signal transduction, nuclear mRNA transport, and modulation of protein-nucleic acid interactions.[13] Type I methyltransferase activity has been found in various tissues and cell types. It catalyzes the asymmetrical dimethylation and monomethylation of arginine residues in various proteins that are involved in signal transduction events (eg, TIS21 immediate-early gene product[33] or the intracellular domain of the interferon-α,β receptor[14]) and in mRNA processing, splicing, and transport into the cytoplasm (eg, hnRNP A1[10]; for a review, see Gary and Clarke[13]). According to data by Abramovich et al,[14] Tang et al,[17] Scott et al,[18] and Katsanis et al,[16] proteins encoded for by PRMT1, PRMT2 (HRMT1L1), PRMT3, and HRMT1L2 genes each contain a catalytic methyltransferase domain and result in the production of ADMA and N^G-monomethyl-L-arginine residues. In contrast, type II *N*-methyltransferase activity has not been purified to homogeneity, and its catalytic subunit has not yet been cloned.[17] Type II *N*-methyltransferase symmetrically dimethylates and monomethylates arginine residues in myelin basic protein in cerebral tissue[33] and therefore is unlikely to be involved in the effects observed in the present study.

The present data show that the gene expression of protein arginine *N*-methyltransferases is increased by nLDL or oxLDL in a concentration-dependent manner. Although the effects of varying concentrations of nLDL and oxLDL on mRNA expression of the 4 isoforms varied slightly, the overall effect was consistent between both cell types and showed a mean 2- to 3-fold elevation. The activation of gene expression by nLDL or oxLDL was blocked by the intracellular antioxidant PDTC, suggesting that redox-regulated mechanisms may underlie this effect. All 4 isoforms exert type I *N*-methyltransferase activity (ie, they mediate asymmetrical dimethylation and monomethylation of arginine residues). This finding is in accordance with our functional data that show ADMA, but not SDMA, levels were elevated in the presence of LDL. Furthermore, the 2- to 3-fold elevation of PRMT mRNA in the presence of nLDL corresponded well to the ≈3-fold increase in radioactively labeled ADMA during incubation with ^{14}C-labeled *S*-adenosylmethionine. The incubation experiments with methionine also showed a clear product preference for ADMA, but not for SDMA, of the methyltransferase activity present in endothelial cells. Taken together, our findings suggest that the gene expression and enzyme activity of protein arginine *N*-methyltransferases are increased in the presence of nLDL and oxLDL and may each contribute in part to increased ADMA elaboration in hypercholesterolemia. These findings can explain previous observations that cultured human endothelial cells release mainly ADMA and less SDMA.[6,7] A greater elevation in ADMA than in SDMA plasma levels has also been found in cholesterol-fed rabbits,[4] in hypercholesterolemic humans in vivo,[2] and in atherosclerotic human subjects[5] and hypertensive patients.[29] In contrast, a greater elevation in SDMA levels is observed in chronic renal failure[34] and in neurological disease.[35] Taken together with our present data, these findings suggest a differential regulation of ADMA and SDMA formation and metabolism/excretion in physiological and pathophysiological states.

A metabolic pathway selective for degradation of ADMA has recently been characterized. The enzyme dimethylarginine dimethylaminohydrolase (DDAH) selectively hydrolyzes ADMA, but not SDMA, to form L-citrulline and dimethylamine.[36] Reduced metabolism might therefore be an alternative explanation for the selective modulation of ADMA release by endothelial cells. However, preliminary evidence from our laboratory suggests that DDAH activity is unchanged in the presence of high homocyst(e)ine levels (R.H. Böger, unpublished observation), and therefore the modulation of DDAH activity may not explain the present results. Recent evidence suggests that oxidative stress induced by oxLDL or by tumor necrosis factor-α reduces DDAH activity.[30] Oxidative stress is increased in advanced stages of atherogenesis and thus may contribute to the elevation of ADMA concentration and subsequent reduced biological activity of endothelium-derived NO in this disease.

In conclusion, the present data suggest that ADMA is formed in human endothelial cells by *S*-adenosylmethionine–dependent protein-arginine *N*-methyltransferases. Although the functional significance of protein arginine *N*-methylation is still incompletely understood, our present data suggest an important regulatory role of this pathway for one of the products that is released during hydrolytic protein turnover: ADMA. This molecule functions as an endogenous inhibitor of NO synthase and may thereby play an important role in endothelial function associated with the initiation and progression of vascular disease. Increased gene expression of protein arginine *N*-methyltransferases with subsequently enhanced methyltransferase activity may be a novel mechanism by which ADMA concentration is elevated in hypercholesterolemia.

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