Inhibition of 3-Hydroxy-3-methylglutaryl (HMG)-CoA Reductase Blocks Hypoxia-mediated Down-regulation of Endothelial Nitric Oxide Synthase*

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Hypoxia induces vasoconstriction, in part, by down-regulating endothelial cell nitric oxide synthase (ecNOS) expression. Previous studies indicate that 3-hydroxy-3-methylglutaryl-coenzyme A (HMG CoA) reductase inhibitors improve endothelium-dependent relaxation by increasing ecNOS activity. To determine whether HMG CoA reductase inhibitors can prevent hypoxia-mediated down-regulation of ecNOS function and expression, human endothelial cells were exposed to hypoxia (3% O2) in the presence of HMG CoA reductase inhibitors simvastatin and lovastatin for various durations (0–48 h).

Hypoxia decreased ecNOS protein and mRNA levels in a time-dependent manner, resulting in a 4- and 9-fold reduction after 48 h, respectively. In a concentration-dependent manner, simvastatin, and to a lesser extent, lovastatin, prevented the down-regulation of ecNOS expression by hypoxia. Simvastatin-induced changes in ecNOS expression correlated with changes in endothelial NO production and were reversed by treatment with l-mevalonate. Actinomycin D studies revealed that under hypoxic conditions, simvastatin increased ecNOS mRNA half-life from 13 to 38 h. Nuclear run-on studies showed that simvastatin had no effect on repression of ecNOS gene transcription by hypoxia. These results indicate that HMG CoA reductase inhibitors regulate ecNOS function and expression through changes in ecNOS mRNA stability and suggest that treatment with HMG CoA reductase inhibitors may have beneficial effects in patients with hypoxia-mediated pulmonary hypertension.

Pulmonary hypertension is a major cause of morbidity and mortality in individuals exposed to hypoxic conditions (1). Recent studies demonstrate that pulmonary arterial vessels from patients with pulmonary hypertension have impaired release of endothelium-derived relaxing factor or nitric oxide (NO) (2, 3). Indeed, individuals with pulmonary hypertension demonstrate reduced levels of endothelial cell nitric oxide synthase (ecNOS)1 expression in their pulmonary vessels and benefit clinically from inhalation NO therapy (4, 5). Conversely, mutant mice lacking ecNOS gene or newborn lambs treated with the ecNOS inhibitor, Nω-monomethyl-L-arginine (LNMA), develop progressive elevation of pulmonary arterial pressures and resistance (6, 7). We and others have shown that hypoxia causes pulmonary vasoconstriction via inhibition of ecNOS expression and activity (8–10). Hence, hypoxia-mediated down-regulation of ecNOS may lead to the vasoconstrictive and structural changes associated with pulmonary hypertension.

Clinical trials with 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitors have shown that a reduction in serum cholesterol level is correlated with improved endothelium-dependent relaxation in atherosclerotic vessels (11, 12). The HMG CoA reductase inhibitors lower serum cholesterol levels by blocking the hepatic conversion of HMG CoA to l-mevalonate in the cholesterol biosynthetic pathway (13). Although the mechanism by which HMG CoA reductase inhibitors restore endothelial function has been almost exclusively attributed to the lowering of serum cholesterol levels (14), little is known whether HMG CoA reductase inhibitors can restore endothelial function under non-hypercholesterolemic conditions. We hypothesize that HMG CoA reductase inhibitors can increase ecNOS activity and improve endothelium-dependent relaxation under hypoxic conditions via effects on endothelial rather than hepatic HMG CoA reductase. Thus, the purpose of this study is to determine whether inhibition of endothelial HMG CoA reductase can modulate hypoxia-mediated down-regulation of ecNOS expression and activity.

EXPERIMENTAL PROCEDURES

Materials—All standard culture reagents were obtained from JRH Biosciences (Lenexa, KS). Actinomycin D, 2,3-diaminonaphthalene, and l-mevalonate were purchased from Sigma. [α-32P]PCTP (3000 Ci/mmol) and [α-32P]UTP (800 Ci/mmol) were supplied by NEN Life Science Products. LNMA was obtained from Calbiochem. The antibody detection kit (Enhanced Chemiluminescence) and the nylon nucleic acid (Hybond) and protein polyvinylidene difluoride transfer membranes were purchased from Amersham Corp. Simvastatin and lovastatin were obtained from Merck Sharp and Dohme. Since endothelial cells lack lactonases to effectively process simvastatin and lovastatin to their active forms, these HMG CoA reductase inhibitors were chemically activated by alkaline hydrolysis before their use, as described previously (15).

Cell Culture—Human endothelial cells were harvested using Type II collagenase (Worthington) as described previously (16). Cells of less than three passages were grown to confluence in a culture medium containing Medium 199, 20 mM HEPES, 50 μg/ml endothelial cell growth factor (Collaborative Research Inc., Bedford, MA), 100 μg/ml heparin sulfate, 5 mM L-glutamine (Life Technologies, Inc.), 5% fetal calf serum (Hyclone, Logan, UT), and an antibiotic mixture of 100 units/ml penicillin, 100 μg/ml streptomycin, 1.25 μg/ml Fungizone. For all experiments, the endothelial cells were grown to confluence before any treatment conditions. In some experiments, cells were pretreated with actinomycin D (5 μg/ml) for 1 h before treatment with HMG CoA reductase inhibitors.

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1 The abbreviations used are: ecNOS, endothelial cell nitric oxide synthase; LNMA, Nω-monomethyl-L-arginine; HMG CoA, 3-hydroxy-3-methylglutaryl coenzyme A; TNF, tumor necrosis factor.

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Exposure to Hypoxia—Confluent endothelial cells grown in 100-mm culture dishes were treated with HMG CoA reductase inhibitors and then placed without culture dish covers in humidified air-tight incubation chambers (Billups-Rothenberg, Del Mar, CA). The chambers were gassed with 20 or 3% O2, 5% CO2, and balanced nitrogen for 10 min before placing the chambers. The culture dishes were maintained in a 37 °C incubator for various durations (0–48 h) and found to have less than 2% variation in O2 concentration, as described previously (9). Cellular confluence and viability were determined by cell count, morphology, and trypan blue exclusion.

Northern Blotting—Equal amounts of total RNA (10–20 µg) were separated by 1.2% formaldehyde-agarose gel electrophoresis, transferred overnight onto Hybond nylon membranes by capillary action, and baked for 2 h at 80 °C before prehybridization. Radiolabeling of human full-length ecNOS cDNA (16) was performed using random hexamer priming, [α-32P]CTP, and Klenow (Pharmacia). The membranes were hybridized with the probes overnight at 45 °C in a solution containing 50% formamide, 5 × SSC (1 × SSC, 0.15 m NaCl and 15 mM sodium citrate), 2.5 X Denhardt’s solution, 25 mM sodium phosphate buffer (pH 6.5), 0.1% SDS, and 250 µg/ml salmon sperm DNA. All Northern blots were subjected to stringent washing conditions (0.2 × SSC, 0.1% SDS at 65 °C) before autoradiography with intensifying screen at −80 °C for 24–72 h. RNA loading was determined by either rehybridization with human glyceraldehyde-3-phosphate dehydrogenase cDNA probe or by ethidium bromide staining of 18 S and 28 S ribosomal RNA on the nylon membranes.

Western Blotting—Cellular proteins were prepared and separated on SDS/polyacrylamide gel electrophoresis as described (9). Immunoblotting was performed using a murine monoclonal antibody to human ecNOS (1:400 dilution; Tandesduction Laboratories, Lexington, KY). Immunodetection was accomplished using a sheep anti-mouse secondary antibody (1:4,000 dilution) and the enhanced chemiluminescence (ECL) kit (Amersham Corp). Autoradiography was performed at 23 °C, and the appropriate exposures were quantitated by densitometry.

In Vitro Transcription Assay—Confluent endothelial cells (5 × 104 cells) were treated with simvastatin (1 µM) in the presence of 20 or 3% O2 for 24 h. Nuclei were isolated and in vitro transcription was performed as described previously (9). Equal amounts (1 µg) of each in vitro transcribed, denatured full-length human ecNOS, human β-tubulin (ATTC number 37855), and linearized PGEM-3z cDNA were vacuum-transferred onto nitrocellulose membranes using a slot blot apparatus (Schleicher & Schuell). Hybridization of radiolabeled mRNA transcripts to the nitrocellulose membranes was carried out at 45 °C for 48 h in a buffer containing 50% formamide, 5 × SSC, 2.5 × Denhardt’s solution, 25 mM sodium phosphate buffer (pH 6.5), 0.1% SDS, and 250 µg/ml salmon sperm DNA. The membranes were then washed with 1 × SSC, 0.1% SDS for 1 h at 65 °C before autoradiography for 72 h at −80 °C. Band intensities were subjected to analyses by laser densitometry.

Assay for ecNOS Activity—The ecNOS activity was determined by a modified nitrate assay as described previously (9, 17). Briefly, endothelial cells grown in phenol-free medium were exposed to either 20 or 3% O2 for 24 h. The activity of ecNOS was assessed by measuring the nitrite production by 75% (110 µM) and 14% (1.5 µM) of conditioned medium was mixed with 30 µl of freshly prepared 2,3-diaminonaphthalene (1.5 µM 2,3-diaminonaphthalene in 1 M HCl). The mixture was protected from light and incubated at 20 °C for 10 min. The reaction was terminated with 15 µl of 2.8 M NaOH. Fluorescence of 1-(H)-naphthotriazole was measured with excitation and emission wavelengths of 365 and 450 nm, respectively. Standard curves were constructed with known amounts of sodium nitrite. Nonspecific fluorescence was determined in the absence of LNA (5 µM). Previous studies with nitrate reductase indicate that the nitrite to nitrate concentration in the medium was approximately 5:1 and that this ratio did not vary with exposure to 20 or 3% O2 concentration (9).

Data Analysis—Band intensities from Northern and nuclear run-on assay blots were analyzed densitometrically by the National Institutes of Health Image Program (18). All values are expressed as mean ± S.E. compared with controls and among separate experiments. Paired and unpaired Student’s t tests were employed to determine the significance of changes in ecNOS activity and densitometric measurements. A significant difference was taken for p values less than 0.05.

RESULTS

Cell Culture—Relatively pure (>98%) human saphenous vein endothelial cell cultures were confirmed by their morphological features (i.e. cuboidal, cobblestone, contact-inhibited) using phase-contrast microscopy and immunofluorescent staining with antibodies to Factor VIII (data not shown). There were no observable adverse effects of HMG CoA reductase inhibitors, l-mevalonate, or hypoxia on cellular morphology and viability. However, higher concentrations of simvastatin (>15 µM) or lovastatin (>50 µM) caused cytotoxicity after 36 h, and therefore, were not used. Otherwise, cellular confluence and viability as determined by trypan blue exclusion were maintained for all treatment conditions described.

Effects of HMG CoA Reductase Inhibitors on ecNOS Activity—The activity of ecNOS was assessed by measuring the LNMA-inhibitable nitrite accumulation from human endothelial cells. Experiments were performed three times in duplicate. *, p < 0.05 compared with untreated condition.

FIG. 1. Effect of hypoxia alone or in combination with simvastatin (1 µM) or l-mevalonate (400 µM) on LNMA-inhibitable nitrite production from human endothelial cells. Experiments were performed three times in duplicate. *, p < 0.05 compared with untreated condition.

Effect of hypoxia alone or in combination with simvastatin (1 µM) or l-mevalonate (400 µM) on LNMA-inhibitable nitrite production from human endothelial cells. Experiments were performed three times in duplicate. *, p < 0.05 compared with untreated condition.

In a concentration-dependent manner, treatment with simvastatin (1 µM) not only completely reversed the down-regulation of ecNOS by hypoxia but resulted in a 3-fold increase in ecNOS activity over basal activity (14 ± 0.5 nmol/500,000 cells/24 h, p < 0.05). This up-regulation of ecNOS activity was attenuated by the addition of l-mevalonate (400 µM) (9.6 ± 1.3 nmol/500,000 cells/24 h, p < 0.05). Interestingly, simvastatin (1 µM) alone up-regulated nitrite production 5-fold (30 ± 4.5 nmol/500,000 cells/24 h, p < 0.01), which was completely blocked by l-mevalonate (400 µM) (8.6 ± 2.9 nmol/500,000 cells/24 h, p < 0.05).

Effect of HMG CoA Reductase Inhibitors on ecNOS Protein Levels—We and others have previously shown that hypoxia decreases ecNOS protein expression (9, 19). Compared with normoxia (20% O2), exposure to hypoxia (3% O2) resulted in a 46% and 75% reduction in ecNOS protein levels after 24 and 48 h, respectively (p < 0.01, n = 3) (Fig. 2A). Treatment with HMG CoA reductase inhibitor, simvastatin (1 µM), increased ecNOS protein levels by 2-fold after 24 h of normoxia (210 ± 18%, p < 0.05, n = 3) and completely reversed the decrease in ecNOS protein levels after 24 h of hypoxia (110 ± 10%, p > 0.05) compared with untreated normoxia (n = 3).

Interestingly, treatment with simvastatin (1 µM) for 48 h resulted in not only a reversal of hypoxia-mediated decrease in ecNOS protein levels but also caused a significant increase in ecNOS protein levels above base line (160 ± 13%, p < 0.05 compared with untreated normoxia, n = 3).

In a concentration-dependent manner, treatment with simvastatin attenuated the hypoxia-mediated decrease in ecNOS protein levels after 48 h (Fig. 2B). At higher concentrations of...
simvastatin (1 and 10 μM), ecNOS protein levels were upregulated to 160 ± 13% and 220 ± 21% above basal levels (p < 0.01, n = 3). Co-treatment with l-mevalonate (400 μM) significantly blocked the simvastatin-induced increase in ecNOS protein levels after 48 h (35 ± 3% above basal levels, p < 0.01, n = 3). Treatment with l-mevalonate alone, however, produced minimal effects on basal ecNOS protein levels in untreated cells exposed to hypoxia (25 ± 4% above basal levels, p < 0.05, n = 3). Simvastatin that was not chemically converted to its active form had no effect on ecNOS expression or activity (data not shown).

Similarly, another HMG CoA reductase inhibitor, lovastatin, also prevented the hypoxia-mediated decrease in ecNOS protein levels in a time- and concentration-dependent manner (Fig. 3). Because lovastatin has a higher IC50 value for HMG CoA reductase compared with that of simvastatin (19), little if any reversal of ecNOS protein levels was observed after 24 h of exposure to hypoxia in the presence or absence of 1 μM of lovastatin (62 ± 7% versus 54 ± 4%). However, after 48 h of exposure to hypoxia, 1 μM lovastatin not only completely reversed the down-regulation of ecNOS protein levels but also increased ecNOS protein levels to 130 ± 9% above basal levels (p < 0.05, n = 3). With higher concentrations of lovastatin (10 μM), ecNOS protein levels were increased to 170 ± 13% that of basal levels (p < 0.01, n = 3). Again, l-mevalonate (400 μM) alone had no effects on ecNOS protein levels under hypoxic conditions but in combination with lovastatin almost completely blocked the effects of the lovastatin-induced increase in ecNOS protein expression (26 ± 23 and 37 ± 3% that of basal levels, respectively). These results indicate that simvastatin- and lovastatin-mediated increases in ecNOS protein expression are mostly likely due to the inhibition of endothelial HMG CoA reductase activity.

**Effects of HMG CoA Reductase Inhibitors on ecNOS mRNA Levels**—To determine whether changes in ecNOS protein levels are due to changes in ecNOS steady-state mRNA levels, we performed Northern blotting on endothelial cells exposed to normoxia and hypoxia in the presence or absence of simvastatin (1 μM) and lovastatin (10 μM). Simvastatin alone increased ecNOS mRNA levels by 340 ± 24% (p < 0.01, n = 3). Exposure of endothelial cells to hypoxia reduced ecNOS mRNA levels by 70 ± 2 and 88 ± 4% after 24 and 48 h, respectively (Fig. 4A). Co-treatment with simvastatin not only completely reversed the hypoxia-mediated decrease in ecNOS mRNA levels but increased ecNOS mRNA levels to 200 ± 12 and 530 ± 30% those of basal levels after 24 and 48 h, respectively (p < 0.01, n = 3). Similarly, lovastatin (10 μM) alone increased ecNOS message by 350 ± 27 and 410 ± 21% under hypoxic and normoxic conditions, respectively (p < 0.01, n = 3) (Fig. 4B). Neither simvastatin nor lovastatin caused any significant change in G-protein αs and β-actin mRNA levels under normoxic or hypoxic conditions (data not shown). These results indicate that the effects of HMG CoA reductase inhibitors are relatively selective in terms of their effects on ecNOS mRNA expression.

**Effects of HMG CoA Reductase Inhibitors on Tumor Necrosis Factor (TNF)-α-mediated Decrease in ecNOS Expression**—Previous studies have shown that cytokines such as TNF-α or interleukin-1 decrease ecNOS mRNA expression (19, 21). To determine whether HMG CoA reductase inhibitors can also prevent cytokine-mediated decrease in ecNOS expression, we treated endothelial cells with TNF-α (10 ng/ml) in the presence or absence of simvastatin (1 μM). Treatment with TNF-α caused a 48 ± 5% reduction in ecNOS protein levels after 24 h (p < 0.01, n = 3) (Fig. 5). Co-treatment with simvastatin completely reversed the TNF-α-mediated decrease in ecNOS protein. Treatment with simvastatin alone increased ecNOS
protein levels by 30 ± 10% over basal levels (p < 0.05, n = 3). These effects of simvastatin were blocked by co-treatment with \( \text{l}-\text{mevalonate} \) (data not shown).

**Effects of HMG CoA Reductase Inhibitors on ecNOS mRNA Half-life**—The half-life of ecNOS mRNA was determined in the presence of actinomycin D (5 \( \mu \text{g/ml} \)). Hypoxia shortened the half-life of ecNOS mRNA from 28 ± 4 to 13 ± 3 h (Fig. 6). Treatment with simvastatin (1 \( \mu \text{M} \)) increased ecNOS half-life to 38 ± 4 and 46 ± 4 h under hypoxic and normoxic conditions, respectively (p < 0.05 for both, n = 3). These results suggest that HMG CoA reductase inhibitors prevent the hypoxia-mediated decrease in ecNOS expression by stabilizing ecNOS mRNA.

**Effects of HMG CoA Reductase Inhibitors on ecNOS Gene Transcription**—Nuclear run-on assays showed that hypoxia caused an 85 ± 8% decrease in ecNOS gene transcription (p < 0.01, n = 3) (Fig. 7). Treatment with simvastatin (1 \( \mu \text{M} \)) did not produce any significant effect on hypoxia-mediated decrease in ecNOS gene transcription (83 ± 6% decrease in ecNOS gene transcription, p > 0.05 compared with hypoxia alone). Furthermore, simvastatin alone produced a minimal increase in ecNOS gene transcription under normoxic conditions (20 ± 5% increase in ecNOS gene transcription, p < 0.05 compared with normoxia control).

Preliminary studies using different amounts of radiolabeled RNA transcripts demonstrate that under our experimental conditions, hybridization was linear and nonsaturable. The density of each ecNOS band was standardized to the density of its corresponding \( \beta\)-tubulin band (relative intensity). To exclude the possibility that changes in \( \beta\)-tubulin gene transcription are caused by hypoxia or simvastatin, another gene, glyceraldehyde-3-phosphate dehydrogenase, was included on each of the nuclear run-on blots. Similar relative indices were obtained when ecNOS gene transcription was standardized to glyceraldehyde-3-phosphate dehydrogenase gene transcription (data not shown). The specificity of each band was determined by the lack of hybridization to the nonspecific pGEM cDNA vector.

**DISCUSSION**

We have shown that HMG CoA reductase inhibitors increase ecNOS expression and prevent hypoxia-mediated down-regulation of ecNOS activity. The mechanism(s) responsible for the increase in ecNOS expression by HMG CoA reductase inhibitors involves post-transcriptional ecNOS mRNA stabilization. There was no effect of HMG CoA reductase inhibitors on ecNOS gene transcription. The findings that co-treatment with \( \text{l}-\text{mevalonate} \) reversed the effects of HMG CoA reductase inhibitors on ecNOS expression suggest that endothelial HMG CoA reductase is an important negative regulator of ecNOS expression. These results are consistent with our finding that simvastatin has a lower IC\(_{50}\) value compared with that of lovastatin for HMG CoA reductase more potently blocks the hypoxia-mediated decrease in ecNOS expression (20).

Despite extensive studies with HMG CoA reductase inhibitors, the biological mechanism(s) involved in their clinical benefits remains unclear. Although improvements in ecNOS activity have been attributed to the lowering of serum cholesterol levels, recent studies suggest that HMG CoA reductase inhibitors may have direct effects on the vascular wall that are independent of serum cholesterol levels (22, 23). Indeed, one of the earliest recognizable benefits after treatment with HMG CoA reductase inhibitors is the normalization of endothelium-dependent relaxation in atherosclerotic coronary arteries before significant lowering of serum cholesterol levels (24). In our study, the effects of HMG CoA reductase inhibitors on ecNOS expression were not due to changes in extracellular cholesterol levels, since all of the endothelial cells were exposed to the same cholesterol concentration. Thus, the clinical applications of HMG CoA reductase inhibitors may well extend beyond hypercholesterolemia and atherosclerosis but also to other pathological conditions where ecNOS activity is found to be diminished, such as in hypoxia-mediated pulmonary hypertension (2, 8).

The effects of HMG CoA reductase inhibitors on ecNOS expression, however, were not specific to hypoxia, since simvastatin produced similar effects in other conditions that are known to destabilize ecNOS mRNA such as interleukin-1 (19), TNF-\( \alpha \) (21), and oxidized low density lipoprotein.\(^2\) Indeed, treatment with simvastatin alone increased ecNOS expression via prolongation of ecNOS mRNA half-life. However, stabiliza-

\(^2\) U. Laufs, V. La Fata, and J. K. Liao, unpublished observation.
tion of ecNOS mRNA by simvastatin was relatively specific, since simvastatin did not prolong the half-life of other constitutively expressed genes such as the G-protein α subunit or β-actin. It is not known, however, how inhibition of endothelial HMG CoA reductase stabilizes ecNOS mRNA. One possibility is that the mevalonate or its downstream lipid derivative stimulates proteins that bind to a sequence motif (AUUUA) in the ecNOS mRNA 3'-untranslated region, which is known to mediate mRNA destabilization via protein-mRNA interaction (25). Another possibility is that ecNOS mRNA stability is cell cycle-dependent. Indeed, synchronization of cell cycle arrest by lovastatin in fibroblasts has been shown to increase the expression of other genes such as p21<sup>Cip1</sup> by post-transcriptional mechanisms (26). Thus, it is interesting to speculate whether HMG CoA reductase inhibitors stabilize ecNOS mRNA indirectly by regulating the cell cycle of vascular endothelial cells.

Inhibition of endothelial I-levalarone synthesis by HMG CoA reductase inhibitors may have many important biological consequences in addition to their effects on cholesterol biosynthesis. For example, metabolism of I-levalarone can yield a series of isoprenoid compounds, including farnesyl, geranylgeranyl, and dolichol derivatives (13). These derivatives allow for the covalent attachments and trafficking of membrane proteins (27). Farnesylation is necessary for the anchoring of G-proteins such as ecNOS (28). Farnesyltransferase is necessary for the synthesis of glycoproteins and may modulate ecNOS activity via effects on membrane fluidity and cell growth (30). Thus, the up-regulation of ecNOS expression via inhibition of endothelial HMG CoA reductase may be mediated by factors resulting from a reduction in I-levalarone metabolism other than cholesterol biosynthesis.

The effect of hypoxia on ecNOS expression, however, remains somewhat controversial. Studies of rats exposed to chronic hypoxia demonstrate normal or increased expression of ecNOS in the pulmonary arterial endothelium (31). Another study showed that hypoxia increases ecNOS promoter activity (32). Yet, considerable evidence including the findings in this study suggests that hypoxia reduces endothelial NO production due to the level of ecNOS expression (8–10, 19). Indeed, we have found a strong correlation between decreases in ecNOS expression and reduction in ecNOS activity under hypoxic conditions. Several possible explanations could account for these reported discrepancies. First, hypoxia may affect other cell types such as macrophages and vascular smooth muscle cells in the pulmonary vasculature, which could indirectly influence ecNOS expression. Second, the duration of hypoxia and subsequent hemodynamic changes associated with hypoxic-mediated vasoconstriction may indirectly affect ecNOS expression <em>in vitro</em>. Third, the cellular sources of ecNOS during hypoxia may come from sources other than the pulmonary endothelium such as the bronchial epithelium, which may be up-regulated rather than down-regulated by hypoxia (33, 34). Finally, hypoxia-mediated increases in ecNOS promoter activity as reported by a recent study may not be physiologic, since the ecNOS promoter used may not contain all of the cis-acting regulatory element(s) (32). When the more definitive studies were performed using <em>in vitro</em> transcription or nuclear run-on assay, our findings were in agreement with that of earlier studies showing that hypoxia represses rather than induces ecNOS gene transcription (9, 10).

In summary, by preventing the down-regulation of ecNOS expression and activity, HMG CoA reductase inhibitors may prove to be useful agents in treating chronically hypoxic individuals with progressive pulmonary hypertension. The findings of this study, therefore, may have important clinical implications since hypoxia-mediated pulmonary hypertension is a major cause of morbidity and mortality in individuals living in high altitudes (1). Further studies, however, are required to determine the mechanism by which I-levalarone or its metabolites destabilizes ecNOS mRNA.