Serotonin transporter protein in pulmonary hypertensive rats treated with atorvastatin

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1Department of Anesthesia and Critical Care, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts; 2Department of Anesthesiology, Vrije University Medical Center, Amsterdam, The Netherlands; 3Department of Anesthesiology and Intensive Care Medicine, University of Leipzig Medical Faculty, Leipzig, Germany; and 4Department of Anesthesia and Critical Care, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts

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Ludi S, Trump S, Schmitz V, West J, McMurtry IF, Mutlak H, Christians U, Weimann J, Kaisers U, Steudel W. Serotonin transporter protein in pulmonary hypertensive rats treated with atorvastatin. Am J Physiol Lung Cell Mol Physiol 293: L630–L638, 2007. First published June 15, 2007; doi:10.1152/ajplung.00110.2006.—HMG-CoA-reductase inhibitors (statins) influence lipid metabolism and have pleiotropic effects. Several statins reduce various forms of pulmonary hypertension (PH) in animal models. The relationship between atorvastatin and expression of serotonin transporter protein (5-HTT) remains unknown. This study focused on the effects of atorvastatin on the course of monocrotaline (MCT)-induced PH and its relation to 5-HTT expression. Male Sprague-Dawley rats were challenged with MCT with or without subsequent daily oral treatment with 0.1, 1, and 10 mg/kg of atorvastatin for 28 days. Over the 4-wk course, the progression of PH was followed by transthoracic echocardiography [pulmonary artery pressure was assessed by pulmonary artery flow acceleration time (PAAT), an estimate reciprocal to pulmonary artery pressure], and, at the end of the 4-wk course, invasive right ventricular pressure, right ventricular weight, quantitative morphology, and 5-HTT expression were measured. MCT caused significant PH as early as 7 days after injection. Atorvastatin treatment increased PAAT and reduced right ventricular pressure, right ventricular hypertrophy, and vascular remodeling over the 4-wk course. MCT challenge was associated with increased pulmonary vascular 5-HTT expression, and atorvastatin treatment reduced the 5-HTT expression. MCT-induced PH over the course of 4 wk can be easily followed by transthoracic echocardiography, and atorvastatin is effective in reducing the PH. Atorvastatin’s effects are associated with a decrease of 5-HTT expression.

monocrotaline; HMG-CoA-reductase inhibitor, 5-HTT, 5-HT

MEDIAL HYPERTROPHY of pulmonary arteries is the most consistent pathological finding in pulmonary hypertension (PH) (34). There is evidence that the medial hypertrophy is due to smooth muscle proliferation (35). In many forms of human primary and secondary PH, the hyperplasia of pulmonary artery smooth muscle cells is attributed to the overexpression of the serotonin transporter (5-HTT) (37).

Serotonin (5-HT) mediates cell signaling by interacting with several subtypes of 5-HT receptors and by internalization through the 5-HTT (2, 17, 33, 39, 65). Mitogenic and hypertrophic responses of smooth muscle cells to 5-HT are believed to be due at least partly to the action of the 5-HTT (9, 29). Both mitogenesis and cell hyperplasia following 5-HT may cause the activation of small GTPases, in particular Rac, the generation of reactive oxygen species (ROS) via NADPH activation, and the activation of the kinases MEK and ERK, as well as the transcription factor GATA-4 further downstream (28, 56, 61). The central role of 5-HTT in PH was underlined by the observation that patients with idiopathic pulmonary artery hypertension had an increased expression of 5-HTT in association with a marked enhancement of proliferative growth in pulmonary artery smooth muscle cell populations in response to 5-HT (12), but to our knowledge, not in response to other growth factors. Experimental data in rats and mice support the hypothesis that altered expression of 5-HTT plays a role in the pathogenesis of PH (7, 16); mice overexpressing 5-HTT develop PH even in the absence of other stimuli (32).

HMG-CoA-reductase inhibitors (statins) exhibit potent anti-proliferative and anti-inflammatory effects besides their cholesterol-lowering effects (31, 58). In recent years, changes of serotonergic pathways in the central nervous system have been observed as a consequence of statin administration (62) and were linked to the expression and activity of the 5-HTT (47, 52). Therefore, it seems quite possible that statins have similar effects on cell populations such as pulmonary artery smooth muscle cells. In different models of PH, the salutary effects of statin treatment were shown, but it was generally linked to alterations in the nitric oxide pathways. For example, in a rat model of PH induced by the combination of monocrotaline (MCT) administration and unilateral pneumectomy, simvastatin attenuated the severity of PH (44, 45). More recently, similar results were reported in hypoxic PH (14). In three independent animal studies using hypoxia to induce PH, the beneficial effects of statins have been related to effects on nitric oxide-mediated pathways. Laufs et al. (27) described inhibition of eNOS downregulation by statins, Nishimura et al. (44) reported increased eNOS expression, and Murata et al. (42) linked the beneficial effects of statins to protection through eNOS activity at a posttranscriptional level. Whereas these studies suggested that increased NO synthase expression and/or activity were beneficial, Girgis et al. (14) reported unchanged eNOS expression during simvastatin treatment. These authors concluded that the prevention of posttransla-
tional lipid modification of small G proteins (e.g., Rac) by statins might have been more important for their findings.

More recently, it was questioned if the effects of statins on PH are effects of all drugs of this pharmacological class, because there is evidence that different statins act differently in PH (48). It was reported by Rakotoniaina et al. (48) that atorvastatin in contrast to pravastatin could not prevent MCT-induced PH, although both equally increased the expression of eNOS.

To our knowledge, the effects of atorvastatin on the expression of 5-HTT have not been studied before. We used atorvastatin since it seems to have the most profound effects on vascular remodeling and smooth muscle proliferation (4, 5, 46). We first studied the effects of atorvastatin on MCT-induced PH in rats and subsequently investigated its influences on the expression of 5-HTT in pulmonary artery smooth muscle cells.

METHODS

Animals and Treatment

Experiments were performed in male Sprague-Dawley rats (age, 8–10 wk; body wt, 280–320 g). Animal protocols were approved by the University of Colorado at Denver and Health Sciences Center Committee on Animal Research, and animal care was conducted in accordance with the NIH guidelines for ethical animal research (Guide for the Care and Use of Laboratory Animals, National Institutes of Health publication No. 85-23, revised 1996). For the hemodynamic measurement procedures, rats were anesthetized with ketamine (40 mg/kg ip) and xylazine (5 mg/kg ip). On day 0, after baseline measurements, PH was induced using MCT (see below). Atorvastatin or vehicle control (skim milk or atorvastatin dissolved in skim milk) was administered by oral gavage daily from day 1 to day 28. On days 0, 7, 14, 21, and 28, transthoracic echocardiography was performed (see below). On day 28, invasive hemodynamic measurements were obtained, and after euthanasia with pentobarbital (100 mg/kg), lung as well as heart tissues were harvested.

Induction of Pulmonary Hypertension

PH was induced on day 0 by a single dose of MCT (60 mg/kg sc; Sigma-Aldrich, St. Louis, MO).

Dose-Dependent Effects of Atorvastatin

Rats were randomly assigned to eight treatment groups (n = 6 rats/group): 1) control (vehicle only); 2) atorvastatin, 0.10 mg·kg⁻¹·day⁻¹; 3) atorvastatin, 1.0 mg·kg⁻¹·day⁻¹ c; 4) atorvastatin, 10.0 mg·kg⁻¹·day⁻¹; 5) MCT + vehicle; 6) MCT + atorvastatin, 0.10 mg·kg⁻¹·day⁻¹; 7) MCT + atorvastatin, 1.0 mg·kg⁻¹·day⁻¹; and 8) MCT + atorvastatin, 10.0 mg·kg⁻¹·day⁻¹. PH was evaluated by echocardiographically determined pulmonary artery acceleration time (PAAT), by invasive measurement of right ventricular systolic pressure (RVSP) on day 28, and by assessment of the degree of vascular muscularization on day 28.
Effects of Atorvastatin on 5-HTT

Right ventricular heart weight in relation to left ventricular and septal weight [RV/(LV+SI)] and expression of 5-HTT were only determined for the most effective dose of atorvastatin.

Echocardiographic Measurements

Transthraxic echocardiography was performed using a 10-MHz ultrasound probe with a Vivid FiVe System (General Electrics Vingmed Ultrasound, Horton, Norway). Echocardiographic data were analyzed with Echopac 6.3.6 software (General Electrics Vingmed Ultrasound). Ejection fraction, shortening fraction, left ventricular end-diastolic diameter, cardiac output, and PAAT were obtained as previously described (21, 49, 57, 64). A decreased PAAT corresponds to an increased pulmonary artery pressure or RVSP (21, 49, 57, 64).

All echocardiographic measurements were performed in triplicate by one investigator and averaged.

Invasive Hemodyamic Measurements

To measure RVSP, a microtip transducer (1.4 Fr; Millar Instruments, Houston, TX) was inserted into the right jugular vein and advanced into the right ventricle. Systolic and diastolic pressures were recorded at closed chest and analyzed using Hemodyn v1.1 software (Hugo Sachs Electronics, March-Hugstetten, Germany).

Lung and Heart Tissue

After measurement of RVSP, the chest was opened and the left lung immediately removed and frozen in liquid nitrogen. The right lung was perfused with buffered formalin. The heart was then removed and dissected, and the free right ventricular wall, left ventricle, and septum were weighed.

Immunohistochemistry

Paraffin-embedded right lung tissue was cut into 5-μm-thick lung sections and subsequently immunostained with an antibody against α-smooth muscle actin (α-SMA) (A2547; Sigma-Aldrich) and 5-HTT (sc-1458; Santa Cruz Biotechnology, Santa Cruz, CA) as described elsewhere (22, 23, 60).

Western Blotting

Expression of 5-HTT in lung tissue was measured by Western blotting using an antibody against 5-HTT (Santa Cruz Biotechnology). After homogenization on ice in Tris-HCl buffer, lung tissue (30 μg of protein) was separated by SDS-PAGE and then transferred to nitrocellulose membranes. Immunodetection was performed with the primary antibody (dilution, 1:500) after incubation in blocking solution. The secondary antibody (donkey anti-goat IgG-HRP; Santa Cruz Biotechnology; dilution, 1:3,000) was then applied after washing, and thereafter, ECL Plus detection (Amersham, Piscataway, NJ) was performed.

RT-PCR

Primers were designed using GenBank sequences and the PerkinElmer ABI Primer Express program: sequence 5’ to 3’, GTCGAGGCTGCAAGACAGT (forward); and sequence 5’ to 3’, TGGCAAAGAACGTGGATGC 1273 R (reverse). Each primer (all are species specific) was searched against Basic Local Alignment Search Tool to ensure that it did not match any known gene, aside from that for which it was designed. The primers were designed specifically for quantitative RT-PCR to produce products of comparable size. RNA was made using a Qiagen RNeasy mini kit (Qiagen, Valencia, CA). cDNA was made using Superscript II RT with oligo(dT)12–18 primers (both from Invitrogen, Carlsbad, CA) from this RNA. For screening gels, PCR was carried out in a GeneAmp Sequence Detection System 5700 (Perkin Elmer, Norwalk, CT) using 40 cycles of 95–60°C PCR with a 10-min 95°C initial soak. For quantitative PCR, the same equipment was used, but the fluorescent indicator Sybrgreen was intercalated to allow real-time light detection. Each sample was tested individually for the housekeeping gene hypoxanthine guanine phosphoribosyl transferase (HPRT) and 5-HTT. Linear increases in fluorescence were confirmed, and the cycle number at detection was expressed relative to that for detection of HPRT. Each measurement was made in triplicate and averaged, with three individual replicate experiments used for statistical analysis (66).

Degree of Muscularization

The degree of pulmonary vascular muscularization was measured as previously reported (22). Wall structure, external diameter (ED), and size of 50 alveolar vessels per animal were determined and recorded, and the wall thickness and ED of muscular and partially muscular vessels were measured. Percent wall thickness (%WT) was calculated as \( \frac{2 \times 100 \times WT}{ED} \).

![Fig. 2. Right ventricular systolic pressure (RVSP); CTR, control; MCT, monocrotaline; AT, atorvastatin; #: \( P < 0.05 \); *: \( P < 0.001 \); (n = 6 rats per group).](http://ajplung.physiology.org/)
Statistical Analysis

All data are shown as means ± SD unless otherwise indicated. Differences between groups were analyzed using one-way ANOVA. Post hoc analysis was performed using the Sidak method (51). Differences in groups were compared using paired \( t \)-tests. A \( P \) value < 0.05 was considered significant. Statistical calculations were performed using SPSS 14.0 software (SPSS, Chicago, IL).

RESULTS

Echocardiographic-Determined PAAT

PAAT did not change in control animals over the course of 28 days.

<table>
<thead>
<tr>
<th>Vessel Type</th>
<th>Control</th>
<th>MCT</th>
<th>MCT + AT 0.1 mg·kg(^{-1})·day(^{-1})</th>
<th>MCT + AT 1.0 mg·kg(^{-1})·day(^{-1})</th>
<th>MCT + AT 10.0 mg·kg(^{-1})·day(^{-1})</th>
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<tbody>
<tr>
<td>Muscularized</td>
<td>24.2 ± 5.7</td>
<td>41.2 ± 6.4(^*)</td>
<td>42.6 ± 9.6(^*)</td>
<td>30.8 ± 12.4</td>
<td>28.3 ± 6.4(^#)</td>
</tr>
<tr>
<td>Partially muscularized</td>
<td>18.8 ± 2.4</td>
<td>32.4 ± 6.1(^*)</td>
<td>31.9 ± 7.7(^*)</td>
<td>24.7 ± 8.5</td>
<td>22.8 ± 5.9(^#)</td>
</tr>
<tr>
<td>Combined</td>
<td>20.9 ± 3.6</td>
<td>37.2 ± 4.2(^*)</td>
<td>37.5 ± 8.5(^*)</td>
<td>29.7 ± 10.9</td>
<td>25.8 ± 9.6(^#)</td>
</tr>
</tbody>
</table>

Percent wall thickness of fully muscularized vessels, partially muscularized vessels, and the combination of both. MCT, monocrotaline; AT, atorvastatin. \(^*\) \( P < 0.001 \) vs. control; \(^#\) \( P < 0.05 \) vs. MCT (\( n = 6 \) rats per group).

\( MCT \) exposure only. PAAT decreased from 27.5 ± 3.1 ms to 20.4 ± 4.3 ms on day 7 (\( P = 0.005 \)) and to 15.3 ± 1.6 ms on day 28 (\( P < 0.001 \)) in \( MCT \)-challenged rats, consistent with PH.

\textbf{Atorvastatin, 0.1 mg·kg\(^{-1}\)·day\(^{-1}\).} PAAT was not different over the 28-day time course in rats that were treated with 0.1 mg·kg\(^{-1}\)·day\(^{-1}\) \textit{atorvastatin} after the initial \( MCT \) challenge compared with rats that were only exposed to \( MCT \) (data not shown).

\textbf{Atorvastatin, 1 and 10 mg·kg\(^{-1}\)·day\(^{-1}\).} Rats treated with daily doses of 1 and 10 mg/kg \textit{atorvastatin} had a significantly lower PAAT at day 14 compared with baseline (23.7 ± 4.1 ms)

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{image.png}
\caption{Representative smooth muscle \( \alpha \)-actin staining (red); control (A); monocrotaline (B); monocrotaline + atorvastatin 0.1 mg·kg\(^{-1}\)·day\(^{-1}\) (C); monocrotaline + atorvastatin 1.0 mg·kg\(^{-1}\)·day\(^{-1}\) (D); and monocrotaline + atorvastatin 10.0 mg·kg\(^{-1}\)·day\(^{-1}\) (E). Bars equal 20 \( \mu \)m.}
\end{figure}
vs. 30.0 ± 4.4 ms and 24.5 ± 5.0 vs. 30.5 ± 2.9 ms, P = 0.005 and P = 0.013, respectively), but PAAT was still above the values of rats that did not receive atorvastatin after MCT challenge (18.6 ± 3.3 ms, P = 0.006).

On day 28, PAAT in MCT-exposed rats that received daily doses of 10 mg·kg\(^{-1}\) atorvastatin (20.6 ± 4.4 ms) was above the values obtained from MCT-challenged rats (15.3 ± 2.2 ms, P < 0.001) but significantly lower in comparison with control rats that also received 10 mg·kg\(^{-1}\)·day\(^{-1}\) atorvastatin (P < 0.001, Fig. 1).

**Summary of echocardiographic findings.** The most important echocardiographic finding was that atorvastatin influences PAAT (and therefore, the degree of PH) over the 28-day course of treatment. Fourteen days of daily doses of 1 and 10 mg atorvastatin caused a clear PAAT increase (and reciprocally, a decrease of estimated pulmonary artery pressure). After 21 days of treatment with atorvastatin, there may be a continuing trend, but this could not be statistically confirmed. After 28 days of daily atorvastatin treatment with 10 mg·kg\(^{-1}\) but not 1 mg·kg\(^{-1}\), the benefit was again visible and statistically supported.

**RVSP**

RVSP was obtained invasively at the end of the 28-day treatment course, and control rats that did not receive MCT but different doses of atorvastatin (0, 0.1, 1, and 10 mg·kg\(^{-1}\)·day\(^{-1}\), respectively) had equal effects on RVSP (Fig. 2A). Rats challenged with MCT had a significantly increased RVSP compared with their controls (P < 0.001). Treatment of MCT rats with atorvastatin showed a significant, dose-dependent decrease of RVSP compared with rats that did not receive atorvastatin (P < 0.001; Fig. 2B).

**Pulmonary Vascular Morphology**

Pulmonary arterioles and small pulmonary arteries of control animals showed only slight staining with α-SMA. α-SMA presence was not affected by different atorvastatin doses in control rats without PH. Percent wall thickness of fully muscularized vessels ranged between 21 ± 5% and 24 ± 6% (not significant). Percent wall thickness increased to 41 ± 6% (P < 0.001) with MCT. Atorvastatin treatment decreased percent wall thickness in a dose-dependent fashion compared with rats that were challenged with MCT but did not receive atorvastatin (Table 1, Fig. 3). Daily doses of 10 mg·kg\(^{-1}\) atorvastatin after MCT challenge reduced the percent wall thickness from 41 ± 6% (MCT) to 28 ± 6% (MCT and 10 mg·kg\(^{-1}\)·day\(^{-1}\) atorvastatin, P = 0.038).

Based on the echocardiographic, hemodynamic, and morphological data, we assumed that the daily atorvastatin administration of 10 mg·kg\(^{-1}\) was most effective and proceeded using this dose for the subsequent experiments.

**RV/(LV+S)**

No differences of RV/(LV+S) were observed between control rats and control rats treated with 10 mg·kg\(^{-1}\)·day\(^{-1}\) atorvastatin. In MCT-challenged rats (not receiving atorvastatin treatment), RV/(LV+S) was 0.57 ± 0.11 compared with animals that were not challenged (0.35 ± 0.08; P < 0.001). MCT-exposed rats that were treated with daily doses of 10 mg·kg\(^{-1}\) atorvastatin for 28 days had a significantly lower RV/(LV+S) than animals challenged with MCT alone (0.41 ± 0.90 vs. 0.57 ± 0.11; P < 0.001, Fig. 4).

**PCR**

In whole lung tissue homogenates, 28 days after MCT challenge, no significant differences between groups were seen for mRNA levels of 5-HTT (Fig. 5).

**Western Blotting**

In whole lung tissue homogenates, 28 days after MCT challenge, expression of 5-HTT increased. In contrast, treatment with atorvastatin decreased the expression of 5-HTT independent of whether the animals received MCT (Fig. 6).

**Immunohistochemistry**

Some 5-HTT staining, mostly in subendothelial layers, was seen in pulmonary vascular smooth muscle cells of control animal lungs. MCT caused a distinct increase in 5-HTT staining in the medial and adventitial layer. This 5-HTT staining was almost totally prevented by atorvastatin treatment (Fig. 7).

**DISCUSSION**

We examined the effects of atorvastatin on MCT-induced PH and, in particular, on the expression of 5-HTT. We found a dose-dependent reduction of PH, which was paralleled by a clear reduction of 5-HTT protein in pulmonary arterial smooth muscle cells. The effects of MCT on the pulmonary artery pressure over time were followed using a noninvasive method, transthoracic echocardiography. As soon as 7 days after induction of PH, we observed that atorvastatin (1.0 mg·kg\(^{-1}\)·day\(^{-1}\)) reduced the progression of PH, and that at a dose of 10.0 mg·kg\(^{-1}\)·day\(^{-1}\) atorvastatin attenuated PH over the course of 28 days. PAAT (an echocardiographic estimate of pulmonary artery pressure) increased, and invasively measured RVSP and right ventricular hypertrophy were significantly reduced by atorvastatin treatment in MCT-challenged rats.

![Fig. 4. Right heart weight in relation to left ventricular and septal weight (RV/(LV+S))](http://ajplung.physiology.org/)
MCT has to be bioactivated in liver cytochrome P-450s (CYP) to MCT pyrrole, which is responsible for the MCT-induced pulmonary hypertension (24). Since atorvastatin is a CYP substrate as well, there might be a possibility that the shown effects are related to impairment of MCT metabolism. In humans, atorvastatin is solely metabolized by cytochrome P-450 (CYP) 3A4 and is, therefore, a strong inhibitor of CYP3A4 (30). In contrast to humans, rats do not have CYP3A4 (8, 38). While in female Sprague-Dawley rats statins are metabolized by CYP3A2, it seems that in male Sprague-Dawley rats statins are metabolized by CYP3A2, it seems that in male Sprague-Dawley rats statins are predominantly metabolized by CYP2C11 (18–20). Since MCT is activated to MCT pyrrole (MCTP) by CYP3A isozymes, but not CYP2C11 (24, 50), it is unlikely that the effects of atorvastatin on MCT-induced PH are inhibitory effects of atorvastatin on the activation of MCT. This is further supported by the fact that the half-life of MCT in rats is rather short, and only small quantities of MCTP are necessary for its pulmonary toxicity (13). After subcutaneous injection, all MCT is activated and, including its metabolites, cleared from the body within 24 h (40, 41, 54). Since we allowed 24 h

Fig. 5. Expression of the serotonin transporter mRNA; relative quantities on four lungs.

Fig. 6. Expression of the serotonin transporter protein (5-HTT); representative Western blots of 5-HTT and β-actin; relative densitometry on five lungs; *P = 0.025

Fig. 7. Serotonin transporter (5-HTT; dark brown) stained lung sections; A: control, small amounts of 5-HTT in the subendothelium; B: MCT cause an increased staining of 5-HTT primarily in the medial/adventitial margin; C: the combination of MCT and atorvastatin (10.0 mg·kg⁻¹·day⁻¹) decreased 5-HTT staining in the medial/adventitial margin.
between the MCT challenge and the beginning of the atorvastatin treatment, probably all of the initially injected MCT was already metabolized or excreted before the atorvastatin treatment was begun.

Using chronic hypoxia as the stimulus to induce PH, Girgis et al. (14) reported that simvastatin (20 mg·kg⁻¹·day⁻¹ ip) reduced PH in rats. In a rat model combining left pneumonectomy and MCT challenge to induce severe PH, Nishimura and colleagues (44) observed that orally administered simvastatin (2 mg·kg⁻¹·day⁻¹ po) clearly attenuated PH. Fluvastatin also reduced hypoxia-induced PH at a dose of 1 mg·kg⁻¹·day⁻¹ po (42). Our results were overall in agreement with these studies (14, 42, 44), which used different approaches to induce PH and different statins; however, the authors mainly addressed endothelial effects of statins, such as eNOS expression and activity.

In a study comparing the effects of pravastatin and atorvastatin on MCT-induced PH, both statins showed the same effects on the expression of eNOS, but treatment with atorvastatin had, in contrast to pravastatin and in contrast to our results, no significant effect on lowering RVSP (48). Therefore, mechanisms other than regulation of eNOS expression have to be involved in the statin’s effect on PH.

We are not aware that a relationship between statin’s effects on the serotonin pathway, especially the serotonin transporter protein, has been studied in the pulmonary circulation.

We observed that MCT-induced PH increased the expression of 5-HTT in smooth muscle cells of the pulmonary arterial circulation. These effects were reversed by using atorvastatin at a dose of 10 mg·kg⁻¹·day⁻¹.

It appears that 4-wk treatment with atorvastatin decreased expression of 5-HTT even in the absence of PH, but the reduction was more prominent in PH.

Even if we could not directly show how atorvastatin decreased the expression of 5-HTT, it is clear from the mRNA data that it seems to be a posttranslational process, since mRNA levels were the same in all groups. It is known that statins disrupt the N-glycosylation of membrane-targeted proteins (53, 55). The N-glycosylation of 5-HTT does not alter its activity but seems to protect 5-HTT from degradation (3). Therefore, one might speculate that atorvastatin prevented the N-glycosylation of 5-HTT, leading to a higher turnover. The high turnover might have resulted in a net decrease of 5-HTT protein amount (26).

There is some evidence that 5-HTT expression is increased in MCT-induced PH. Guignabert et al. (16) showed an early and sustained increase of 5-HTT mRNA and protein levels after MCT challenge. In chronic hypoxia, the correlation between 5-HTT and PH is less clear. While Eddahibi et al. (9, 10) reported increased 5-HTT transcription and expression in smooth muscle cells of the pulmonary circulation, other studies describe decreased expression of 5-HTT (32, 39, 63). Congenital absence of 5-HTT renders mice less prone to hypoxia-induced PH (10), whereas an increased expression of 5-HTT is a common observation in various forms of human PH (11, 12, 37).

Additional evidence for an important role of 5-HTT in PH has been derived from data showing that selective inhibition of 5-HTT prevents PH. 5-HTT inhibition using citalopram or fluoxetine attenuated PH in chronically hypoxic mice (36). Fluoxetine also prevented the development of PH in MCT-challenged rats (16). In general, there seems to be agreement that a higher expression of 5-HTT is associated with a higher pulmonary arterial pressure, and the inhibition of this pathway reduces PH.

An additional possible mechanism for the beneficial effects of atorvastatin on PH might be the inhibition of the RhoA/ROCK pathway. It has been observed that Rho-kinase-mediated pathways play a central role in the pathogenesis of PH (1). The inhibition of the Rho-kinase by fasudil improved the PH substantially (1, 43). Statins inhibit the Rho-kinase pathway (6, 15, 25), which raises the possibility that the effects of atorvastatin might have been at least partly due to inhibition of RhoA and/or Rac1.

One limitation of our study is that we did not distinguish between the role of vasoconstriction or remodeling in PH. Stenmark and McMurtry (59) recently emphasized the importance of relaxation of pulmonary vessels prior to evaluation of their luminal obstruction by neomuscularization. They suggested the administration of a Rho-kinase inhibitor before permanent fixation of the lung to allow maximal relaxation of the vasculature.

In summary, we conclude that atorvastatin prevents MCT-induced PH. Atorvastatin treatment in pulmonary hypertension is associated with the downregulation of HTT expression in pulmonary vascular smooth muscle cells, reduced pulmonary artery pressure, reduced vascular remodeling, and reduced right ventricular hypertrophy. Further details of the exact molecular mechanism need to be addressed in future studies.

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SEROotonin TRANSPORTER IN PULMONARY HypERTENSION


