19,20-EpDPE, a bioactive CYP450 metabolite of DHA monoacylglyceride, decreases Ca$^{2+}$ sensitivity in human pulmonary arteries

Caroline Morin,1,2 Samuel Fortin,1 and Eric Rousseau2

1SCF Pharma, Sainte-Lace, and 2Department of Physiology and Biophysics, Faculty of Medicine and Health Sciences, Université de Sherbrooke, Sherbrooke, Quebec, Canada

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MORIN C, Fortin S, Rousseau E. 19,20-EpDPE, a bioactive CYP450 metabolite of DHA monoacylglyceride, decreases Ca$^{2+}$ sensitivity in human pulmonary arteries. Am J Physiol Heart Circ Physiol 301: H1311–H1318, 2011. First published August 5, 2011; doi:10.1152/ajpheart.00380.2011.—The aim of this study was to investigate the effect of docosahexaenoic acid monoacylglyceride (MAG-DHA) on human pulmonary arterial tension. Tension measurements on pulmonary arterial tissues demonstrated that MAG-DHA reduced U-46619-induced tone, which is highly sensitive to the H-1152 inhibitor. Results also showed that MAG-DHA treatments decreased RhoA activity levels, which in turn inactivated the Rho-kinase pathway, leading to a reduction in U-46619-induced Ca$^{2+}$ sensitivity of permeabilized pulmonary artery smooth muscle cells. According to the mechanical responses assessing U-46619-induced Ca$^{2+}$ sensitivity in the absence or presence of 3 μM MAG-DHA, MAG-DHA plus 1 μM N-methylsulfonyl-6-(2-propargyloxyphenyl) hexanamide (MS-PPOH, a cytochrome P-450 epoxyxygenase inhibitor) and 300 nM 19,20-epoxydocosapentaenoic acid (a cytochrome P-450 epoxyxygenase-dependent DHA metabolite), our data suggest that the MAG-DHA is metabolized in a bioactive epoxymetabolite. This epoxyeicosanoid in turn decreases active tone and Ca$^{2+}$ sensitivity of smooth muscles cells through an inhibition of the Rho-kinase pathway. Together, these data provide primary evidence regarding the mode of action of MAG-DHA in human pulmonary arteries and suggest that this compound may be of pharmacological interest in patients with pulmonary hypertension to generate intracellular bioactive metabolites.

19,20-epoxydocosapentaenoic acid; docosahexaenoic acid derivative; hypertension; pulmonary artery; Rho-kinase; cytochrome P-450

PULMONARY ARTERIAL HYPERTENSION (PAH) is a rare but dramatic disease characterized by a progressive increase in pulmonary vascular resistance (PVR) and pulmonary arterial pressure (PAP), leading to right ventricle hypertrophy and death (14, 32). Although advances in the understanding of disease development and treatment have been achieved, the pathogenesis of PAH is still not clearly understood (15). Among promising targets recently identified is the Rho-kinase pathway (21). Rho kinase regulates a variety of cellular functions including motility, proliferation, apoptosis, contraction, and gene expression (17, 21). Rho kinase is considered to be a major determinant of arterial tone, through its essential role in the regulation of Ca$^{2+}$ sensitivity of the contractile machinery in smooth muscle cells (17, 33). Recent pharmacological studies suggest that activation of the small G protein RhoA and its target Rho kinase is a critical shared mechanism in the pathogenesis of PAH (21).

In vivo, potent effects of treatment with Rho-kinase inhibitors (Y-27632 or fasudil) have been demonstrated in several animal models of PAH (1, 5, 27). Furthermore, acute intravenous administration of low-dose fasudil has been shown to reduce PVR and PAP in patients with PAH (8, 9, 16).

Arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) can be metabolized by cytochrome P-450 (CYP450) enzymes into several classes of oxygenated metabolites (6, 10). Several of these CYP450-derived eicosanoids are also known for their ability to modulate vascular (31) and airway smooth muscle tone (24, 25). These epoxymetabolites have been proposed as putative endothelium- and epithelium-derived hyperpolarizing factors, which activate large-conductance Ca$^{2+}$-activated K$^+$ (BKCa) channels, inducing hyperpolarization and partial relaxation of vascular smooth muscle (VSM) and bronchial smooth muscle cells (18, 25, 36). The CYP450-dependent DHA metabolites include the epoxydocosapentaenoic acid (EpDPE) regioisomers 4,5-, 7,8-, 10,11-, 13,14-, 16,17-, and 19,20-EpDPE (4, 6, 22). Several epoxyeicosanoids have been identified in lung tissues, including 212, 2C8, 2C9, and 1B1 (18). Recent studies have demonstrated that CYP450 epoxyeicosanate metabolites of DHA display potent vasodilatory activities on coronary arteries and have been suggested to be more potent than epoxyeicosatrienoic acids and EPA in activating BKCa channels (18, 36).

The objective of the present study was to investigate the effect of DHA monoacylglyceride (MAG-DHA) on human pulmonary arterial tone. Complementary approaches were used to perform tension measurements, to assess putative changes in Ca$^{2+}$ sensitivity, and to determine the activation of RhoA and the phosphorylation level of other regulating proteins in human pulmonary arteries (HPAs). Herein, we report the first evidence that MAG-DHA induces the production of 19,20-EpDPE through the activity of CYP450 epoxyeicosanate enzymes in HPAs. This epoxyeicosanoid decreases the activation of RhoA, leading to a reduction in Ca$^{2+}$ sensitivity and active tone induced by U-46619 on HPAs.

MATERIALS AND METHODS

Isolation and culture of human pulmonary tissues. The study was approved by our institution’s Ethics Committee (Protocol number CRC 05-088-S1-R2), and consent was obtained from each patient. Human lung tissues were obtained from 16 patients undergoing surgery for lung carcinoma. Following lobectomy and transport in sterile physiological saline solution, lung samples, distant from the malignant lesion, were dissected by the pathologist. The absence of tumoral infiltration was retrospectively established in all tissue sections by pathological analysis. Tissue samples were immediately placed in Krebs solution, previously bubbled with 95% O$_2$-5% CO$_2$ (pH 7.4) at 22°C and then immediately transported to a level 2 culture room. Rings of similar weight and length (inner diameter of 0.5–0.8
mm) were microdissected from the same pulmonary artery segment. Arterial rings were placed in individual wells of 24-well culture plates containing DMEM-F12 culture medium (1.5 ml/well), supplemented with 0.3% penicillin (100 IU/ml) and streptomycin (0.1 mg/ml). Explants were placed in a humidified incubator at 37°C under 5% CO2-95% room air for 24 h (25). Explants were untreated (control) or treated with either 0.03 μM U-46619, MAG-DHA (0.01–100 μM), or with 19,20-EpDPE (0.001–10 μM) before pharmacological challenges. For specific experiments, explants were treated with either a selective Rho-kinase inhibitor H-1152 or with N-methylsulfonyl-6-(2-propargyloxyphenyl) hexanamide (MS-PPOH), a CYP450 epoxide nase inhibitor (34).

Isometric tension measurements. The mechanical effects induced by specific agonists and eicosanoids were measured as previously described (25). Passive and active tensions were assessed using transducer systems (Radnoti Glass, Monrovia, CA), coupled to Polyview software (Grass-Astro-Med, West Warwick, RI) for facilitating data acquisition and analysis.

Western blot analysis. Western blots using specific antibodies against RhoA, phospho-myosin phosphatase target protein-1 (p-MYPT1), phospho-myosin light chain (p-MLC), and MLC proteins were performed on homogenate fractions as previously described (24, 25). The immunostaining of the blots were digitized and analyzed with Lab Image software 2.7-2.

Measurement of RhoA activity. RhoA activity was assessed in homogenized tissue samples by pull-down assays using the Rho-binding domain of the Rho effector: Rhotekin, according to manufacturer instructions (RhoA activation assay kit, Cell Biolabs, Cedarlane Laboratories, Burlington, ON, Canada).

Drugs and chemical reagents. 19,20-EpDPE, U-46619, and MS-PPOH were obtained from Cayman Chemical (Ann Arbor, MI). MAG-DHA was obtained from SCF Pharma (Rimouski, QC, Canada). The vehicle was tested separately at the maximal concentration (pCa = −log [Ca2+]), as previously described (15).

RESULTS

Effect of MAG-DHA on pulmonary arterial smooth muscle tension. Experiments were designed to assess the effect of MAG-DHA on the reactivity induced by U-46619, a thromboxane A2/prostanoid receptor agonist, on pulmonary arterial tone. MAG-DHA was synthesized as previously described (7), and the chemical structure is shown in Fig. 1A, inset. Human distal pulmonary arteries were cultured for 24 h in the absence or presence of increasing concentrations of MAG-DHA. The tissues were subjected to 0.6-g basal tone and thereafter challenged with 30 nM U-46619. The cumulative concentration response curve (CCRC) to MAG-DHA (0.01–100 μM) re-
RhoA protein is known to activate Rho kinase, leading to an inhibitory effect of 95% was quantified in the presence of MAG-DHA, to a similar extent (50%) on treated HPAs. Moreover, an inhibitory tension induced by pCa 6.0 in the presence of U-46619 were of respective inhibitory effects of MAG-DHA and H-1152 on HPAs and tissues pretreated with 3 μM MAG-DHA in HPA tissues compared with the response in untreated tissues. The combined addition of 3 μM MAG-DHA and 10 nM H-1152 displays a 94% inhibitory effect on U-46619-induced responses. To evaluate the putative nonspecific effects of MAG-DHA on HPA reactivity, a corn oil monoacylglyceride of similar structure, containing 54% linoleic acid (C18:2n6), 33% oleic acid (C18:1), and 12% palmitic (C16:0), was synthesized and used as a negative control. Analysis of the mean response demonstrated that treatment of HPAs with 3 μM corn oil monoacylglyceride for 24 h had no effect on the reactivity to U-46619, compared with the responses of control tissues. These results confirm that MAG-DHA likely displays specific effects on HPAs.

**Effect of MAG-DHA treatment on the activation of RhoA.**

The selective Rho-kinase inhibitor H-1152 was used to determine whether MAG-DHA could interfere with the activation of the Rho-kinase pathway to explain the observed tension reduction in HPAs. H-1152 (10 nM and 300 nM) was used to treat HPAs; these concentrations represent the IC_{50} value and the maximal inhibitory effect calculated on HPAs precontracted with U-46619 (data not shown). Figure 1B displays the bar histogram of mean tensions induced by either U-46619 in the absence (control) or presence of 10 nM H-1152, 3 μM MAG-DHA alone, or in combination with 10 nM H-1152. Results demonstrated a significant reduction in U-46619-induced tension upon treatment with 10 and 300 nM H-1152 and 3 μM MAG-DHA in HPA tissues compared with the response in untreated tissues. The combined addition of 3 μM MAG-DHA and 10 nM H-1152 displays a 94% inhibitory effect on U-46619-induced responses. To evaluate the putative nonspecific effects of MAG-DHA on HPA reactivity, a corn oil monoacylglyceride of similar structure, containing 54% linoleic acid (C18:2n6), 33% oleic acid (C18:1), and 12% palmitic (C16:0), was synthesized and used as a negative control. Analysis of the mean response demonstrated that treatment of HPAs with 3 μM corn oil monoacylglyceride for 24 h had no effect on the reactivity to U-46619, compared with the responses of control tissues. These results confirm that MAG-DHA likely displays specific effects on HPAs.

**Effect of MAG-DHA treatment on the activation of RhoA.**

To assess the effect of MAG-DHA pretreatments on Ca^{2+} sensitivity, comparative analyses were performed on β-escin-permeabilized human pulmonary arterial rings. Figure 2A illustrates CCRC to free Ca^{2+} concentrations on permeabilized HPA rings obtained from control and treated tissues. When compared with the control (untreated) condition (Fig. 2A, dashed line), an addition of U-46619 to the organ bath enhanced the Ca^{2+} sensitivity to precalibrated Ca^{2+} step increases in HPA explants (Fig. 2A, open circles). However, MAG-DHA pretreatment resulted in a marked inhibitory effect on Ca^{2+} sensitivity (right shift) developed by U-46619-treated explants. Data analysis demonstrated that MAG-DHA treatment induced a shift in EC_{50} values (0.94 ± 0.02 μM) toward higher Ca^{2+} concentrations when compared with untreated tissues challenged with U-46619 (0.13 ± 0.04 μM) (Fig. 2A). However, the difference in Ca^{2+} sensitivity between control HPAs and tissues pretreated with 3 μM MAG-DHA was not significant, with EC_{50} values of 0.65 ± 0.03 and 0.51 ± 0.03 μM, respectively (Fig. 2A). Furthermore, the involvement of the Rho-kinase pathway was evaluated using the pharmacological inhibitor H-1152. Figure 2B displays the mean tension induced by pCa 6.0 in control (untreated) and treated tissues with either 10 nM H-1152 or 3 μM MAG-DHA, as well as after stimulation with 30 μM U-46619 in the absence or presence of either 10 nM H-1152, 3 μM MAG-DHA, or 3 μM MAG-DHA + 10 nM H-1152. Data analysis revealed that the respective inhibitory effects of MAG-DHA and H-1152 on tension induced by pCa 6.0 in the presence of U-46619 were of similar extent (50%) on treated HPAs. Moreover, an inhibitory effect of 95% was quantified in the presence of MAG-DHA and H-1152.

**Effect of MAG-DHA treatment on the activation of RhoA.**

MAG-DHA was not selected for this analysis. The selective Rho-kinase inhibitor H-1152 was used to determine whether MAG-DHA could interfere with the activation of the Rho-kinase pathway to explain the observed tension reduction in HPAs. H-1152 (10 nM and 300 nM) was used to treat HPAs; these concentrations represent the IC_{50} value and the maximal inhibitory effect calculated on HPAs precontracted with U-46619 (data not shown). Figure 1B displays the bar histogram of mean tensions induced by either U-46619 in the absence (control) or presence of 10 nM H-1152, 3 μM MAG-DHA alone, or in combination with 10 nM H-1152. Results demonstrated a significant reduction in U-46619-induced tension upon treatment with 10 and 300 nM H-1152 and 3 μM MAG-DHA in HPA tissues compared with the response in untreated tissues. The combined addition of 3 μM MAG-DHA and 10 nM H-1152 displays a 94% inhibitory effect on U-46619-induced responses. To evaluate the putative nonspecific effects of MAG-DHA on HPA reactivity, a corn oil monoacylglyceride of similar structure, containing 54% linoleic acid (C18:2n6), 33% oleic acid (C18:1), and 12% palmitic (C16:0), was synthesized and used as a negative control. Analysis of the mean response demonstrated that treatment of HPAs with 3 μM corn oil monoacylglyceride for 24 h had no effect on the reactivity to U-46619, compared with the responses of control tissues. These results confirm that MAG-DHA likely displays specific effects on HPAs.

**Effect of MAG-DHA treatment on the activation of RhoA.**

Effect of MAG-DHA treatment on the activation of RhoA. RhoA protein is known to activate Rho kinase, leading to an inhibition of MLC phosphatase, which in turn maintains the phosphorylation of MLC as well as VSM tone (33). Herein, experiments were designed to assess the activity of RhoA and the phosphorylation level of MYPT-1 and MCP in untreated and MAG-DHA-treated HPAs.

RhoA activation assay was performed in homogenized tissue samples by pull-down assays using the Rho-binding domain of the Rho effector rhotekin. Data analyses revealed that 3 and 30 μM MAG-DHA significantly reduced the activity of RhoA, as demonstrated by a lower amount of RhoA coupled to GTP (Fig. 3, A and B). Western blot analysis revealed that U-46619
treatment increased the phosphorylated active form of MYPT-1 and MLC (Fig. 3C). In contrast, the phosphorylation of MYPT-1 and MLC were reduced upon MAG-DHA treatment and essentially normalized when compared with non-treated controls (Fig. 3C). Moreover, an additional reduction of the MYPT-1 and MLC phosphorylation levels were delineated in the presence of H-1152 in MAG-DHA-treated HPAs. Total RhoA, MYPT-1, and MLC staining were constant from one preparation to the other regardless of the experimental conditions (Fig. 3C). Quantitative analysis of identical immunoblot membrane areas were then normalized as a function of total MLC staining in the corresponding fractions. As shown in Fig. 3D, a 24-h pretreatment of HPA explants with 3 μM MAG-DHA significantly reduced p-MLC-to-MLC staining density.

Fig. 3. Effect of MAG-DHA on RhoA activation and on myosin phosphatase target protein-1 (MYPT-1) and myosin light chain (MLC) phosphorylation levels. A: Rho activation assay was performed on HPA homogenates derived from control (untreated) and U-46619 treated in the absence and presence of MAG-DHA. B: quantitative analysis of various GTP-to-RhoA density ratios. Staining densities in the homogenates were expressed as a function of β-actin signals. *P < 0.05, n = 6. C: proteins from distinct homogenates were stained using specific antibodies against the phosphorylated forms of MYPT-1 Thr696 (p-MYPT-1) and MLC Ser19 (p-MLC) as well as the total form of RhoA, MYPT-1, and MLC. Experimental conditions were as follows: control (untreated), 30 nM U-46619 alone and in the presence of 10 nM H-1152, 3 μM MAG-DHA or combined addition of these two compounds. D: quantitative analysis of mean p-MYPT-1-to-MYPT-1 density ratios. Staining densities in the homogenates were expressed as a function of MYPT-1 signals. *P < 0.05; n = 6.
ratio, when compared with the ratio in U-46619-treated tissues (Fig. 3D, n = 6). These results hence correlate with the functional measurements described in Fig. 2, A and B.

Effect of 19,20-EpDPE on HPA tone. Tension measurements were performed on HPAs to evaluate the effect of 19,20-EpDPE, a well-known metabolite of DHA, on active tone. HPAs were cultured for 24 h in the presence of varying concentrations of 19,20-EpDPE (0.001–10 μM) and thereafter challenged with 30 nM U-46619. CCRC to 19,20-EpDPE displayed a concentration-dependent inhibitory effect with an EC50 value of 0.11 ± 0.03 μM (Fig. 4A).

The specific CYP450 epoxygenase inhibitor MS-PPOH was used to minimize the production of epoxymetabolites derived from endogenous ω-6- and ω-3-polyunsaturated fatty acid (PUFA), such as AA and DHA, respectively. It was assumed that MAG-DHA is hydrolyzed by PLA1 and that DHA could be processed by endogenous epoxygenases. To investigate this possibility, comparative analyses were performed to assess the effect of MAG-DHA in the absence or presence of 3 μM MS-PPOH on the reactivity developed by HPAs upon U-46619 stimulation. Figure 4B quantifies the mean responses to U-46619 on control (untreated) and treated HPAs, either in the presence of 3 μM MAG-DHA or 3 μM MAG-DHA plus 1 μM MS-PPOH, or following 300 nM 19,20-EpDPE pretreatment. Mean response values demonstrated that MAG-DHA and 19,20-EpDPE treatments largely decreased the pharmacological responsiveness of HPAs, whereas the addition of MS-PPOH largely blunted the effect of MAG-DHA (Fig. 4B), which would be consistent with CYP450 epoxygenase inhibition and a lower production of epoxy-DHA metabolites (19,20-EpDPE) from exogenously added MAG-DHA. Together, these data suggest that a MAG-DHA metabolite, such as 19,20-EpDPE, endogenously produced by the activity of CYP450 epoxygenase, could mediate the reduction in responsiveness observed upon U-46619 stimulation in HPAs. However, the production of other epoxy- and polyhydroxy-DHA metabolites cannot be ruled out.

Effect of 19,20-EpDPE, a MAG-DHA CYP450 epoxygenase metabolite, on Ca2+ sensitivity. Comparative analyses were subsequently performed on β-escin-permeabilized preparations to measure the Ca2+ sensitivity of MAG-DHA- and 19,20-EpDPE-treated HPAs, either in the absence or presence of 1 μM MS-PPOH. Quantitative analysis of the effect of U-46619 in the presence of a pCa 6.0 on permeabilized preparations obtained from control and pretreated HPAs are shown in Fig. 5A. Data demonstrated that MAG-DHA and 19,20-EpDPE treatments significantly decreased U-46619-induced tension of HPAs, whereas the addition of MS-PPOH largely reduced the effect of MAG-DHA (Fig. 5A), which would be consistent with a lower production of 19,20-EpDPE. Thus, to investigate whether or not these observations were correlated with a change in the status of contractile proteins, the activation of RhoA was assessed in HPAs following MAG-DHA treatment in the absence or presence of MS-PPOH and in 19,20-EpDPE-treated tissues alone. Western blot and quantitative analyses of GTP-RhoA-to-β-actin ratio revealed that MAG-DHA treatment reduced the activity of RhoA induced by U-46619, whereas an increased RhoA activity was observed in the presence of MS-PPOH. Hence, 19,20-EpDPE pretreatment resulted in a reduction of RhoA activity when compared with the activation level detected upon U-46619 treatment (Fig. 5B).

Figure 5C illustrates a functional diagram summarizing the putative mode of action of MAG-DHA and 19,20-EpDPE, its CYP450 epoxygenase metabolite, on intracellular mechanisms involved in U-46619-stimulated HPA tension. The exogenous addition of MAG-DHA leads to the sequential production of DHA and 19,20-EpDPE by the activity of PLA1 and CYP450 epoxygenase, respectively. The epoxyeicosanoid decreases the activity of RhoA, leading to an inhibition of Rho-kinase...
Fig. 5. Effect of 19,20-EpDPE on Ca\(^{2+}\) sensitivity induced by U-46619 in HPAs. A: bar histogram displaying the mean tension induced by pCa 6.0 in untreated tissues and after stimulation with 30 μM U-46619 in control, 3 μM MAG-DHA, 300 nM 19,20-EpDPE, as well as in 3 μM MAG-DHA + 1 μM MS-PPOH-treated HPAs. *P < 0.05; n = 12 for each experimental condition. B: RhoA activity was determined in distinct HPA homogenates using the β-actin as a loading controls. C: quantitative analyses of various GTP-RhoA-to-β-actin density ratios are presented. Mean ratios are representative of 6 independent experiments. *P < 0.05. D: functional diagram summarizing the inhibitory effect of MAG-DHA and 19,20-EpDPE on thromboxane A\(_2\)/prostanoid receptor (TP-R)-increased Ca\(^{2+}\) sensitivity (U-46619 induced) in HPAs. CYP450, cytochrome P-450; MLCP, MLC phosphatase; MLCK, MLC kinase.
activation, which results in a lower Ca\textsuperscript{2+} sensitivity of HPAs (as shown in Fig. 5, A and B).

**DISCUSSION**

**MAG-DHA inhibits activation of the Rho-kinase pathway in HPAs.** The Rho-kinase pathway participates in vasoconstriction elicited by numerous agents involved in PAH, including thromboxane A\textsubscript{2}, endothelin-1, and 5-hydroxytryptamine (11, 12). Rho is a small monomeric GTPase that activates Rho-associated protein kinase which in turn phosphorylates and inhibits MLC phosphatase, leading to prolonged, refractory vasoconstriction. In the present study, our data attest that MAG-DHA, a newly synthesized DHA derivative, or one of its metabolites targets the Rho-kinase pathway to reduce U-46619-induced tension in HPAs. Moreover, we were able to demonstrate that MAG-DHA treatment reduced the activation of RhoA, which in turn inactivated the Rho-kinase pathway, resulting in a reduction in U-46619-induced Ca\textsuperscript{2+} sensitivity of human pulmonary arterial smooth muscle cells. Altogether, these data suggest that MAG-DHA could represent a new pharmacological agent of clinical interest in the management of PAH.

Several studies have demonstrated that the use of Rho-kinase inhibitors reduces PAH in many animal models (26, 30). Y-27632 inhaled at 10–100 mM was shown to reduce mean PAP without altering systemic arterial pressure in a hypoxic rat model of hypertension (29). In the monocrotaline model, fasudil (30 or 100 mg kg\textsuperscript{-1} day\textsuperscript{-1} per os) improved survival, pulmonary hypertension, right ventricular hypertrophy, as well as pulmonary vascular lesions (1). In Fawn-hooded rats exhibiting a raised PAP, inhaled fasudil reduced PAP to 55 mmHg without altering mean systemic arterial pressure (28). In humans, Rho-kinase inhibition with fasudil has been shown to bring about an immediate, albeit modest, reduction in PVR, although this Rho-kinase inhibitor must be administrated by nebulization to avoid systemic hypotension (8, 16). H-1152 was used as a pharmacological tool to assess the involvement of Rho-associated protein kinase and RhoA pathway in the mode of action of thromboxane A\textsubscript{2}/prostanoid receptor agonist and inhibition induced by MAG-DHA. Our results demonstrate that MAG-DHA prevents RhoA activation which might reduce Rho-kinase-mediated MYPT-1 phosphorylation and therefore inhibit Ca\textsuperscript{2+} sensitization of HPAs.

**CYP450, the MAG-DHA epoxygenase metabolite, reduces tension of HPAs.** It is widely accepted that n-3 PUFAs (ω3-PUFA), rich in fish oils, protect against several types of cardiovascular diseases such as myocardial infarction, arrhythmia, atherosclerosis, hypertension, and inflammatory conditions (2, 3, 19). Docosahexaenoic acid (DHA), EPA, or their derivatives might represent active biological components mediating these effects (24). Although the precise cellular and molecular mechanisms underlying these beneficial effects are still uncertain, the protective effects of ω3-PUFA are likely related to their direct effects on VSM cells (13, 23). It has been shown that these ω3-PUFAs activate ATP-sensitive K\textsuperscript{+} and BK\textsubscript{Ca} channels while inhibiting specific types of Ca\textsuperscript{2+} channels (36). These reports suggest that the modulation of VSM cell functions contributes to the beneficial effects of ω3-PUFA in the systemic cardiovascular system. Moreover, in a previous study, we have shown that 17,18-EpETE, an EPA CYP450 epoxygenase metabolite, induced a concentration-dependent relaxation of smooth muscle from distal HPAs which was related to an activation of BK\textsubscript{Ca} and ATP-sensitive K\textsuperscript{+} channels (25). Furthermore, in the present study, our data revealed that the CYP450 epoxygenase-dependent DHA metabolite, 19,20-EpDPE, is likely responsible for the reduction in U-46619-induced Ca\textsuperscript{2+} sensitivity in HPAs, when compared with the mechanical responses observed in the presence of MAG-DHA and MAG-DHA plus MS-PPOH (as shown herein in Figs. 4 and 5). Consequently, the pharmacological prevention of tonic responses triggered in HPAs, as well as the ability of this metabolite to reduce responsiveness, may therefore be of physiological significance in pulmonary hypertension. Moreover, it has recently been demonstrated that DHA and EPA epoxides share and even exceed the ability of AA epoxides to stimulate Ca\textsuperscript{2+}-activated potassium (BK\textsubscript{Ca}) channels (20) and to mediate vasodilatation in canine and porcine coronary microvessels (37). Moreover, it was reported that DHA induces vasodilatation of coronary arteries, this effect being mediated by CYP450 epoxygenase metabolites, resulting in an activation of vascular BK\textsubscript{Ca} channels (35, 36). Further investigations using in vivo models, such as a hypoxic rat model or a monocrotaline model of hypertension would be required to determine the efficacy of MAG-DHA per os treatments in reversing pulmonary hypertension. The identification of CYP450 epoxygenase metabolites using high performance liquid chromatography coupled to tandem mass spectrometry could be useful to explain the effect of MAG-DHA in these in vivo hypertension models.

**Conclusion.** The present data provide relevant evidence regarding the mode of action of MAG-DHA in HPAs. In HPAs, an exogenous addition of MAG-DHA induced the production of 19,20-EpDPE by activity of the CYP450 epoxygenase enzymes. This epoxyeicosanoid metabolite prevented U-46619-induced vasoconstriction through a reduction of the Rho-kinase activation pathway leading to a lower Ca\textsuperscript{2+} sensitivity of HPAs. This MAG-DHA or its bioactive metabolite may hence represent a new and prospective pharmacological compound of low toxicity and medicinal interest in modulating vasoconstriction in patients with pulmonary hypertension.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

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