20-Hydroxyeicosatetraenoic acid is a vasoconstrictor in the newborn piglet pulmonary microcirculation

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Fuloria, Mamta, Delrae M. Eckman, Daniel A. Leach, and Judy L. Aschner. 20-Hydroxyeicosatetraenoic acid is a vasoconstrictor in the newborn piglet pulmonary microcirculation. Am J Physiol Lung Cell Mol Physiol 287: L360–L365, 2004. First published April 9, 2004; 10.1152/ajplung.00358.2003.—20-Hydroxyeicosatetraenoic acid (20-HETE), a cytochrome P-450 metabolite of arachidonic acid, is a vasoconstrictor in the systemic circulation and a vasodilator in the adult pulmonary circulation. Little is known about the vasoactive properties of 20-HETE in the newborn pulmonary circulation. The objectives of this study were to determine the vascular effects of 20-HETE and to explore the signaling mechanism(s) that mediate these effects in newborn pulmonary resistance-level arteries (PRA). Our findings demonstrate that, in contrast to the adult pulmonary circulation where 20-HETE mediates vasodilation, it causes constriction in newborn PRA at resting tone. Furthermore, inhibition of cyclooxygenase (COX) with indomethacin augments 20-HETE-induced constriction. The enhanced constrictor response to 20-HETE under conditions of COX inhibition is abolished in endothelium-disrupted PRA, suggesting that 20-HETE either stimulates endothelium-derived COX to release a counteracting vasodilator or is rapidly metabolized by COX to a less potent vasoconstrictor. 20-HETE-induced constriction is significantly inhibited by blocking calcium-dependent K⁺ (KCa) channels and the thromboxane-PGH₃₂ receptor. Altogether, our data indicate that the vascular actions of 20-HETE are partially mediated via the activation of KCa channels and are significantly modulated by interactions with the COX-prostaglandin pathway.

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20-HETE were dissolved in ethanol; A-23187 was reconstituted in DMSO; and indomethacin was dissolved in 250 mM Na2CO3. In each case, the vehicle had no effect on the vascular reactivity of pulmonary resistance arteries (PRA). Care was taken to protect the SNP solution from light and 20-HETE from light and air.

Animals

All experimental protocols were performed in adherence with the National Institutes of Health guidelines for the use of experimental animals. This study was approved by the Animal Care and Use Committee at Wake Forest University School of Medicine. Newborn piglets were housed in the Wake Forest University School of Medicine Animal Resource Facilities. This facility is maintained by the Department of Comparative Medicine and is fully accredited by the American Association for Accreditation of Laboratory Animal Care.

Isolation of PRA

PRA were isolated from newborn piglets as previously described (1, 8). In brief, piglets (1–4 days old) were killed with an overdose of pentobarbital sodium (75–100 mg/kg ip), heart and lungs were removed en bloc, and PRA (<300 μm diameter, branching order 8–12) were dissected in oxygenated Krebs-Henseleit buffer (118 mM NaCl, 4.8 mM KCl, 1.2 mM MgSO4·7H2O, 1.2 mM KH2PO4, 25 mM NaHCO3, 11 mM dextrose, and 2 mM CaCl2·2H2O; pH 7.4). All side branches were tied with a single fiber of 10-0 braided nylon thread. Subsequently, the arteries were transferred to a well of an arteriograph (Living Systems Instrumentation, Burlington, VT), where they were cannulated at one end, secured with a single strand of suture, gently flushed free of blood, and cannulated at the distal end. The arteriograph was set on the stage of an inverted microscope (Nikon TMS) with a video camera attached to the viewing tube (Sony XC 73). A pressure servo-system connected to the proximal cannula maintained intraluminal pressure at 15–20 mmHg. The vessel image was projected on a television monitor, and the lumen diameter (LD) was continuously measured with a video dimension analysis system (Living Systems Instrumentation).

PRA Study Protocol

After a 30-min equilibration period with a stable baseline LD, PRA were constricted by addition of 50 mM KCl, followed by addition of either Ach (10−5 M) or A-23187 (10−5 M), a calcium ionophore, to demonstrate intact vascular smooth muscle and endothelial function, respectively. Ach, a receptor-mediated endothelium-dependent vasodilator, and A-23187, a receptor-independent but endothelium-dependent vasodilator, were used interchangeably to verify the presence of a functional endothelium in cannulated PRA. Both agonists induce a similar dilation in KCl-constricted PRA, and both Ach and A-23187 induce constriction in endothelium-disrupted PRA (1). Preparations that failed to constrict to KCl and/or to dilate in response to SNP and calcium-free media were excluded from analysis. In our vascular preparation, addition of 50 mM KCl resulted in 38 ± 2% constriction and addition of ACh or A-23187 resulted in a 54 ± 2% dilation response. This was followed by another 30-min equilibration period in which each PRA returned to its stable baseline LD.

Pulmonary vascular effects of 20-HETE and the role of the COX-prostaglandin pathway. We determined the concentration-dependent (10−10–10−7 M) effects of exogenous 20-HETE on resting vascular tone in endothelium-intact cannulated, pressurized PRA. For subsequent experiments, we used 10−7 M 20-HETE, since this concentration induced significant constriction in PRA whereas lower concentrations did not. Furthermore, it has previously been shown that endogenous 20-HETE levels in systemic microvessels are ∼100 nM (9, 22). We determined the role of the COX pathway of AA metabolism in the vascular effects of 20-HETE by comparing the responses of PRA to 20-HETE (10−7 M) in the absence and presence of indomethacin (10−5 M for 30 min), a nonselective COX inhibitor.

Role of the endothelium. To determine whether the vascular actions of 20-HETE are endothelium dependent, we disrupted the endothelium of some PRA by intraluminal perfusion of 2–10 ml of air, followed by a 10-min perfusion with Krebs buffer, as previously described (1, 8, 18). Only air-denuded vessels that constricted with KCl but either failed to dilate or constricted in response to Ach or A-23187 were studied further. Endothelium-disrupted PRA were exposed to exogenous 20-HETE (10−7 M) in the absence and presence of indomethacin (10−5 M for 30 min).

Effect of elevated tone on the vascular response of 20-HETE. To determine the effect of elevated vascular tone on the response to 20-HETE, we preconstricted PRA with either U-46619 (10−7 M) or ET-1 (10−9 M) in the absence and presence of indomethacin (10−5 M), before the exogenous addition of 20-HETE (10−7 M). Both U-46619 and ET-1, a potent pulmonary vasoconstrictor that is independent of the COX-prostaglandin signaling pathway, induced a stable and sustained constriction in this vascular preparation.

Role of thromboxane-PGF2α receptor. The role of the thromboxane-PGFα receptor in 20-HETE-mediated constriction was assessed in both the absence and presence of indomethacin (10−5 M), with the receptor antagonist SQ-29548 (10−5 M). At the completion of these studies, the efficacy of receptor blockade was determined by the addition of the thromboxane mimetic U-46619.

Role of KCa channels. To determine the role of KCa channel inhibition in 20-HETE-mediated vasoconstriction, we pretreated PRA for 30 min with either IBTX (10−7 M), charybdotoxin (CTX, 5 × 10−8 M), or apamin (10−6 M) before the exogenous addition of 20-HETE (10−7 M). IBTX is an inhibitor of large-conductance KCa channels; CTX is an inhibitor of large- and intermediate-conductance KCa channels, whereas apamin inhibits small-conductance KCa channels. These experiments were performed both in the absence and presence of COX inhibition.

Vascular Integrity

At the end of each study, vascular integrity was assessed by demonstrating constriction to KCl (120 mM) and relaxation to SNP followed by superfusion with calcium-free buffer to achieve a maximum LD. PRA that failed to constrict to KCl and/or to dilate in response to SNP and calcium-free media were excluded from analysis.

Data Analysis

The number of piglets studied is represented by n. The responses are expressed as percent change from baseline LD or as absolute change in LD from the U-46619- or ET-1-induced constriction. Data are expressed as means ± SE. Statistical analysis was performed with SPSS 12.0 (Chicago, IL). Data were analyzed by a paired t-test, a one-way ANOVA, or with a repeated-measure model with within-group contrasts, as appropriate. Comparisons between groups were analyzed with a post hoc Tukey’s multiple comparison test at the 0.05 level of significance.

RESULTS

Pulmonary Vascular Effects of 20-HETE and the Role of the COX-Prostaglandin Pathway

20-HETE (10−10–10−7 M) induces a concentration-dependent constriction in newborn PRA. At concentrations <10−7 M, constriction to 20-HETE was small and statistically insignificant, whereas 10−7 M 20-HETE induced a modest constriction in PRA (Fig. 1).

As shown in Fig. 2, in the absence of indomethacin, 10−7 M 20-HETE induced a significant (8 ± 3%) constrictor response in newborn PRA. Vasoconstriction to 20-HETE was significantly augmented in the presence of COX inhibition with
indomethacin (19 ± 2% constriction, LD decreased from 187 ± 14 to 151 ± 11 μm).

Role of the Endothelium

Endothelial disruption abolished 20-HETE-induced constriction both in the absence and presence of indomethacin (Fig. 2).

Effect of Elevated Tone on the Vascular Response to 20-HETE

In the presence of indomethacin, PRA preconstricted with U-46619 (10⁻⁷ M) or ET-1 demonstrated no further constriction or dilation in response to 10⁻⁷ M 20-HETE (Fig. 3, A and B). Similarly, no further change in LD was observed in preconstricted PRA exposed to 20-HETE in the absence of indomethacin (data not shown).

Role of Thromboxane-PGH₂ Receptor

In the absence of indomethacin (10⁻⁵ M), inhibition of the thromboxane-PGH₂ receptor blockade with SQ-29548 (10⁻⁵ M) blocked ~50% of 20-HETE-induced constriction (data not shown). Similarly, in indomethacin-treated PRA, thromboxane-PGH₂ receptor blockade with SQ-29548 significantly blunted 20-HETE-induced constriction, resulting in a constriction that was similar in magnitude to that induced by 20-HETE in the absence of COX inhibition (Fig. 4). In PRA treated with SQ-29548, exogenous addition of U-46619, a thromboxane A₂ mimetic, at the end of the experiment did not result in any further constriction or dilation in response to 20-HETE (n = 9, *P < 0.05 different from baseline).

Role of K₈ Channels

As shown in Fig. 5, in the presence of indomethacin, selective inhibition of large-conductance K₈ channels with IBTX (10⁻⁷ M) inhibited ~50% of 20-HETE-induced constriction. Inhibition of large- and intermediate-conductance
K_{Ca} channels with CTX (5 × 10^{-8} M) or small-conductance K_{Ca} channels with apamin (10^{-6} M) inhibited 90% of the 20-HETE-mediated constriction. In the absence of indomethacin (10^{-5} M), both CTX and apamin inhibited ~50% of 20-HETE-induced constriction (data not shown).

**DISCUSSION**

Our novel findings delineate the vasoactive effects of 20-HETE in resistance-level pulmonary arteries from the newborn piglet. Unlike the adult pulmonary circulation where 20-HETE is a vasodilator, this cp450 4A metabolite of AA causes concentration-dependent vasoconstriction in newborn piglet PRA (Fig. 1). In this respect, the newborn pulmonary vascular response to 20-HETE is more similar to the adult systemic than the adult pulmonary circulation. In contrast to our findings, 20-HETE is a vasodilator in the adult human (2), rabbit (26), and bovine pulmonary arteries (25). Birks and colleagues (2) have shown that 20-HETE relaxes phenylephrine-constricted pulmonary artery rings from adult New Zealand White rabbits. Furthermore, these authors also noted that treatment with 17-octadecynoic acid (17-ODYA), a suicide substrate inhibitor of cp450 enzymes, augments phenylephrine-induced constriction of pulmonary artery rings, suggesting that 17-ODYA inhibits production of a dilator cp450 metabolite of AA (26). More recently, in U-46619- or norepinephrine-precontracted bovine pulmonary arteries, 20-HETE-mediated dilation has been attributed to the release of NO and an increase in intracellular Ca^{2+} in pulmonary artery endothelial cells (25). Several methodological differences distinguish these prior studies in adult pulmonary arteries and our study. Thus it is possible that the size of the pulmonary arteries studied (Birks: 351 ± 26 μm; Zhu: 1–2 mm; current study: 184 ± 12 μm), differences in the vascular preparations (Zhu: pulmonary artery rings mounted on tungsten wires; Birks and the current study: pressurized pulmonary arteries), and/or species differences underlie the opposite effects reported. We considered the possibility that a dilation response to 20-HETE is uncovered only when vascular tone is elevated because preconstriction was a frequent feature of the prior studies. However, as shown in Fig. 3, A and B, no significant change in LD was noted in response to exogenous addition of physiologically relevant concentrations of 20-HETE when newborn PRA were preconstricted with either U-46619 or ET-1. These data indicate that 20-HETE is a vasoconstrictor in the newborn pulmonary circulation at resting tone. An alternative and more plausible explanation is that the response of the pulmonary circulation to 20-HETE is a developmentally regulated process, with a shift from vasoconstriction in the newborn to vasodilation in the adult pulmonary circulation.

Another novel finding is that the constriction response to 20-HETE (10^{-7} M) is significantly augmented in the presence of indomethacin to inhibit COX activity (Fig. 2). One possible mechanism for the unexpected finding of enhanced 20-HETE-induced constriction under conditions of COX inhibition is that 20-HETE is rapidly metabolized by COX to a less vasoactive metabolite. Another possibility is that 20-HETE induces the release of a COX-derived vasodilator, such as prostacyclin (PGI2), that counteracts the constriction induced by 20-HETE. Elimination of the COX-derived dilator unmasks the true potency of 20-HETE as a constrictor in the newborn lung. Interestingly, Birks and colleagues (2) have shown that 20-HETE-induced dilation of human pulmonary arteries is COX dependent since it is abolished by indomethacin. This is consistent with the hypothesis that 20-HETE induces the release of a COX-derived vasodilator, such as PGI2 or PGE2, in the pulmonary circulation of both the adult and the newborn. In contrast, 20-HETE-induced constriction in rat aortic rings is blocked by inhibition of COX, an effect that was only partially endothelium dependent (5, 21). This suggests that in adult systemic conduit vessels, 20-HETE stimulates release of a COX-derived constrictor prostaglandin, most likely thromboxane, from the vascular smooth muscle, endothelium, or both, whereas in adult and newborn pulmonary resistance-level arteries, a dilator prostaglandin from the endothelium is the predominant COX-derived product released by 20-HETE stimulation.
As COX proteins are expressed in both the endothelium and the vascular smooth muscle (VSM), we determined whether the enhanced constriction to 20-HETE in indomethacin-treated PRA was attributable to inhibition of endothelium-derived COX or COX expressed in the VSM. As shown in Fig. 2, we found that the COX-mediated enhancement of 20-HETE-induced vasoconstriction in PRA from newborn piglets was endothelium dependent, since disruption of the endothelium prevented augmentation of the constriction response in the presence of indomethacin. This finding indicates that COX expressed in the pulmonary endothelium is responsible for 20-HETE inactivation or metabolism to a less potent constrictor. Alternatively, COX in the endothelium releases a vasodilator upon stimulation by 20-HETE. These results indicate that a COX isoform, presumably COX-1, expressed in the endothelium and not the VSM of newborn PRA, modulates the vascular actions of 20-HETE. Our data indicate that the endothelium plays a role in 20-HETE-induced constriction in newborn PRA. 20-HETE may induce the release of endothelium-derived constrictors, such as endothelin, isoprostanones, or oxygen-derived free radicals. In the absence of COX inhibition, the vascular action of any endothelium-derived constrictor is balanced by the simultaneous release of dilator prostaglandins from the endothelium. The corollary of these findings is that factors (physiological or pathological) that alter COX activity may greatly influence the response of the newborn pulmonary circulation to 20-HETE.

Our data demonstrating inhibition of 20-HETE-induced vasoconstriction by the thromboxane-PGH₂ receptor antagonist SQ-29548 (Fig. 4) illustrates yet another interaction between the cP450 and the COX pathways of AA metabolism. These findings are similar to those reported in the rat aortic rings (5, 21) and porcine coronary arteries (20) demonstrating inhibition of 20-HETE-induced constriction by inhibitors of the thromboxane receptor. The inhibition of 20-HETE-induced constriction by SQ-29548 suggests that 20-HETE regulates pulmonary vascular tone, at least in part, via activation of the thromboxane-PGH₂ receptor. Whether 20-HETE directly activates the thromboxane-PGH₂ receptor or indirectly results in receptor activation by inducing release of thromboxane is unknown. However, our novel finding that COX inhibition augments 20-HETE-induced vasoconstriction suggests that the dominant prostaglandin product released after 20-HETE addition is a dilator prostaglandin and not a potent constrictor, such as thromboxane.

In the adult systemic circulation, 20-HETE has been shown to inhibit large-conductance K⁺Ca channels (11, 27) and induce an increase in intracellular Ca²⁺ (17). Because the vascular response in the newborn pulmonary circulation is similar to that observed in the adult systemic circulation, we sought to determine the role, if any, of K⁺Ca channels in 20-HETE-induced constriction of PRA. Inhibition of K⁺Ca channels by addition of IBTX (to inhibit large-conductance K⁺Ca channels), CTX (to inhibit large- and intermediate-conductance K⁺Ca channels), or apamin (to inhibit small-conductance K⁺Ca channels) resulted in a small constriction response, suggesting that K⁺Ca channel activity modulates resting pulmonary vascular tone. Each of these three K⁺Ca channel inhibitors will cause membrane depolarization. Subsequent exposure of the depolarized PRA to 20-HETE resulted in a blunted constrictor response (Fig. 5), suggesting that 20-HETE mediates constriction in newborn piglet PRA by inducing VSM depolarization, possibly via inhibition of large-, intermediate-, and/or small-conductance K⁺Ca channels.

In summary, unlike the adult human, rabbit, and bovine pulmonary circulations where it is a dilator, 20-HETE causes constriction of newborn piglet PRA. This constriction is significantly augmented by COX inhibition and abolished by disruption of the endothelium. 20-HETE-induced constriction is blunted by inactivation of K⁺Ca channels and by inhibition of the thromboxane-PGH₂ receptor. Altogether, our data indicate that the vascular actions of exogenous 20-HETE are partially mediated via the activation of K⁺Ca channels and are significantly influenced by interactions with the COX-prostaglandin pathway. We speculate that endogenously produced 20-HETE may modulate resting tone in the newborn pulmonary circulation.

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