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20-Hydroxyeicosatetraenoic Acid is a Vasoconstrictor in the Newborn Piglet Pulmonary Microcirculation

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Abstract:

20-hydroxyeicosatetraenoic acid (20-HETE), a cytochrome P450 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 endothelial-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^+ (K_{Ca}) channels and the thromboxane-PGH_2 receptor. Altogether, our data indicate that the vascular actions of 20-HETE are partially mediated via the activation of K_{Ca} channels and are significantly modulated by interactions with the COX-prostaglandin pathway.

Keywords: cytochrome P450; cyclooxygenase**;** K_{Ca} channels, thromboxane-PGH_2 receptor

Abbreviations:

PRA: pulmonary resistance artery

LD: lumen diameter

U46619: 9, 11-dideoxy-11α, 9α-epoxy-methanoprostaglandin $F_{2\alpha}$

ET-1: endothelin-1

ACh: acetylcholine

cP450: cytochrome P450

17-ODYA: 17-octadecynoic acid

DDMS: N-methylsulfonyl-12,12-dibromododec-11-enamide

COX: cyclooxygenase

NO: nitric oxide

L-NA: nitro-L-arginine

20-HETE: 20-hydroxyeicosatetraenoic acid

IBTX: iberiotoxin

CTX: charybdotoxin

K_{Ca}: calcium-dependent K^+ channels

SNP: sodium nitroprusside

DMSO: dimethylsulfoxide

Introduction

Cytochrome P450 (cP450) metabolites of arachidonic acid (AA) contribute to the regulation of cardiovascular function and arterial blood pressure. In the adult systemic circulation, multiple roles for products of the cP450 pathway have been proposed including endothelium-derived hyperpolarizing factors (3), (19), oxygen sensors (6), (12), 20-(16) and mediators of hypertension and pressure-induced tone (7), (9), (11), (13), (14), (24). In contrast, in the pulmonary circulation, the functional significance of the cP450 pathway of AA metabolism has not been adequately investigated, particularly in the newborn.

20-hydroxyeicosatetraenoic acid (20-HETE), a cP450 4A metabolite of AA, has been shown to play an important role in the regulation of systemic vascular tone. It is a potent constrictor in multiple systemic vascular beds including the cerebral, renal, mesenteric and skeletal arterioles (9), (11), (15), (17), (23). Multiple signaling mechanisms have been implicated in 20-HETE-induced constriction in the systemic circulation including inhibition of large-conductance calcium-dependent K^+ (K_{Ca}) channels (11), (27) and direct activation of voltage-dependent Ca^{2+} channels (10). Activation of a tyrosine kinase in the mitogen-activated protein kinase cascade also contributes to the effects of 20-HETE on K^+ channel activity and vascular tone in the renal circulation of the rat (23). 20-HETE-induced constriction is partially dependent on the presence of the endothelium (4), (20), (21) and is abolished by inhibition of cyclooxygenase (COX) with indomethacin (5), (21) and by the endoperoxide/thromboxane receptor antagonist, SQ-29548 (21). Similarly, in porcine coronary arteries, 20-HETE-induced constriction is partially blocked by inhibition of COX and the thromboxane receptor (20).

Relative to the extensive investigations in the systemic circulation, little is known about the role of cP450 metabolites, including 20-HETE, in the modulation of pulmonary vascular tone, especially in the newborn lung. In contrast to the systemic circulation where 20-HETE is a vasoconstrictor, it has been shown to be a vasodilator in adult human, rabbit and bovine pulmonary arteries (2), (25), (26). In humans, 20-HETE-mediated vasodilation is endothelium and COX-dependent, suggesting that either 20-HETE is metabolized by COX to a vasodilator

metabolite or it stimulates the release of dilator COX metabolites from the endothelium (2). More recently, Yu *et al* have shown that 20-HETE dilates bovine pulmonary arteries by a mechanism that involves an increase in intracellular Ca^{2+} and nitric oxide (NO) release in bovine pulmonary artery endothelial cells (25).

Given the varied but pivotal role of 20-HETE in the regulation of systemic and adult pulmonary vascular tone, the objectives of the present study were to (1) determine the vascular effects of 20-HETE in the newborn piglet pulmonary microcirculation, and (2) explore the signaling mechanism(s) that mediate changes in newborn pulmonary vascular tone in response to 20-HETE.

Methods

Reagents

9, 11-dideoxy-11α, 9α-epoxy-methanoprostaglandin $F_{2\alpha}$ (U46619) was purchased from Calbiochem (San Diego, CA). Iberiotoxin (IBTX) was purchased from Alomone (Jerusalem, Israel) and SQ-29548 from Biomol (Plymouth Meeting, PA). All other chemicals were obtained from Sigma Chemical Co (St. Louis, MO).

Drug stock solutions were prepared as follows: acetylcholine (ACh), apamin and sodium nitroprusside (SNP) were prepared as aqueous solutions. U46619, endothelin-1 (ET-1), SQ-29548 and 20-HETE were dissolved in ethanol; A23187, was reconstituted in dimethylsulfoxide (DMSO); and indomethacin was dissolved in 250 mM Na_2CO_3. In each case, the vehicle had no effect on the vascular reactivity of 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 AAALAC.

Isolation of pulmonary resistance arteries

Pulmonary resistance arteries (PRA) were isolated from newborn piglets as previously described (1), (8). Briefly, piglets (1-4 days old) were sacrificed with an overdose of sodium pentobarbital (75-100 mg/kg i.p.), heart and lungs were removed *en bloc* and pulmonary resistance-level arteries (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 $MgSO_4 \cdot 7H_2O$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 11 mM dextrose, and 2 mM $CaCl_2 \cdot 2H_2O$; 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 mm Hg. The vessel image was projected on a TV monitor and the lumen diameter (LD) was continuously measured using a video dimension analysis system (Living Systems Instrumentation, Burlington, VT).

PRA study protocol

After a 30-minute equilibration period with a stable baseline LD, PRA were constricted by addition of 50 mM KCl, followed by addition of either acetylcholine (ACh; 10^{-5} M) or A23187 (10^{-5} M), a calcium ionophore, to demonstrate intact vascular smooth muscle and endothelial function, respectively. ACh, a receptor mediated endothelium-dependent vasodilator, and A23187, 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 A23187 induce constriction in endothelium-disrupted PRA (1). Preparations that failed to constrict to KCl and/or failed to dilate to either ACh or A23187 were excluded from further study. In our vascular preparation, addition of 50 mM KCl resulted in 38±2% constriction and addition of ACh or A23187 resulted in a 54±2% dilation response. This was followed by another 30-minute 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 while lower concentrations did not. Furthermore, it has previously been shown that endogenous 20-HETE levels in systemic microvessels is approximately 100 nM (9), (22). The role of the COX pathway of AA metabolism in the vascular effects of 20-HETE was determined by comparing the responses of PRA to 20-HETE (10^{-7} M) in the absence and presence of indomethacin (10^{-5} M for 30 minutes), a non-selective COX inhibitor.

Role of the endothelium:

To determine whether the vascular actions of 20-HETE are endothelium-dependent, the endothelium of some PRA was disrupted by intraluminal perfusion of 2-10 mL of air, followed by a 10-minute 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 A23187 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 minutes).

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, PRA were preconstricted with either U46619 (10^{-7} M) or with ET-1 (10^{-9} M) in the absence and presence of indomethacin (10^{-5} M), prior to the exogenous addition of 20-HETE (10^{-7} M). Both U46619 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-PGH_2 receptor:

The role of the thromboxane-PGH_2 receptor in 20-HETE-mediated constriction was assessed both in the absence and presence of indomethacin (10^{-5} M), using 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, U46619.

Role of K_{Ca} channels:

To determine the role of K_{Ca} channel inhibition in 20-HETE-mediated vasoconstriction, PRA were pretreated for 30 minutes with either IBTX (10^{-7} M), CTX ($5X10^{-8}$ M) or apamin (10^{-6} M) prior to the exogenous addition of 20-HETE (10^{-7} M). IBTX is an inhibitor of large conductance K_{Ca} channels; CTX is an inhibitor of large and intermediate conductance K_{Ca} channels whereas apamin inhibits small conductance K_{Ca} 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

"n" represents the number of piglets studied. The responses are expressed as percent change from baseline LD or as absolute change in LD from the U46619 or ET-1-induced constriction. Data are expressed as mean ± SEM. Statistical analysis was performed using SPSS 12.0 (Chicago, IL). Data were analyzed using either a paired t-test, 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 PRAs. At concentrations less than 10^{-7} M, constriction to 20-HETE was small and statistically insignificant whereas 10^{-7} M 20-HETE induced a modest constriction in PRAs (Figure 1).

As shown in Figure 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 μm to 151±11 μm).

Role of the endothelium:

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

Effect of elevated tone on the vascular response to 20-HETE:

In the presence of indomethacin, PRA preconstricted with U46619 (10^{-7} M) or ET-1 demonstrated no further constriction or dilation in response to 10^{-7} M 20-HETE (Figure 3A and

3B). 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-PGH_2 receptor:

In the absence of indomethacin (10^{-5} M), inhibition of the thromboxane-PGH_2 receptor blockade with SQ-29548 (10^{-5} M) blocked approximately 50% of 20-HETE-induced constriction (data not shown). Similarly, in indomethacin-treated PRA, thromboxane-PGH_2 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 (Figure 4). In PRA treated with SQ-29548, exogenous addition of U46619, a thromboxane A_2 mimetic, at the end of the experiment did not result in any further constriction of PRA.

Role of calcium-dependent K^+ (K_{Ca}) channels:

As shown in Figure 5, in the presence of indomethacin, selective inhibition of large conductance K_{Ca} channels with IBTX (10^{-7} M) inhibited approximately 50% of 20-HETE-induced constriction. Inhibition of large and intermediate conductance K_{Ca} channels with CTX ($5X10^{-8}$ 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 approximately 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 (Figure 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 circulations (25). Birks and colleagues have shown that 20-HETE elicits a dose and cyclooxygenase (COX)-dependent dilation in human pressurized small pulmonary arteries (2). Similarly, Zhu and colleagues have observed that 20-HETE relaxes phenylephrine-constricted pulmonary artery rings from adult New Zealand white rabbits (26). 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 U46619 or norepinephrine-preconstricted 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 Figures 3A and 3B, 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 U46619 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 (Figure 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 dilator prostaglandin, such as prostacyclin (PGI_2), 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 have shown that 20-HETE-induced dilation of human pulmonary arteries is COX-dependent since it is abolished by indomethacin (2). This is consistent with the hypothesis that 20-HETE induces the release of a COX-derived vasodilator, such as prostacyclin or prostaglandin E_2, 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, while 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 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 Figure 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 PRAs, modulates the vascular actions of 20-HETE. Our data indicate that the endothelium plays a role in 20-HETE-induced constriction in newborn PRAs. 20-HETE may induce the release of endothelium-derived constrictors, such as endothelin, isoprostanes, or oxygen-derived free

radicals. In the absence of COX inhibition, the vascular action of any endothelial-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-PGH_2 receptor antagonist, SQ-29548, (Figure 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-PGH_2 receptor. Whether 20-HETE directly activates the thromboxane-PGH_2 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 Ca^{2+} (17). Since 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 PRAs. Inhibition of K_{Ca} channels by addition of either 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 (Figure 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 20-HETE is a dilator, it 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-PGH_2 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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Figure Legends:

Figure 1. Cumulative concentration-dependent response of newborn PRAs to 20-HETE (10^{-10}-10^{-7} M). (n=5; *p < 0.05 different from baseline)

Figure 2. Percent constriction from baseline LD by 20-HETE (10^{-7} M) in endothelium-intact (- Indo; n = 5; + Indo; n = 14) and –disrupted (- Indo; n = 4; + Indo; n = 6) PRA. 20-HETE induced significant constriction in newborn PRAs which was significantly augmented in the presence of COX inhibition with indomethacin (10^{-5} M). Disruption of the endothelium abolished 20-HETE-induced constriction both in the absence and presence of indomethacin. (*p < 0.05 different from baseline; #p < 0.05 different from intact (- Indo) group)

Figure 3A. Mean LD of indomethacin-treated (10^{-5} M) PRA preconstricted with U46619 (10^{-7} M) before and after addition of 20-HETE (10^{-7} M). PRA preconstricted with U46619 demonstrated no further constriction or dilation in response to 20-HETE. (n = 9; *p < 0.05 different from baseline)

Figure 3B. Mean LD of indomethacin-treated (10^{-5} M) PRA preconstricted with ET-1 (10^{-9} M) before and after addition of 20-HETE (10^{-7} M). In ET-1 preconstricted PRA, 20-HETE caused no further constriction or dilation. (n = 6; *p < 0.05 different from baseline)

Figure 4. Percent constriction from baseline LD by 20-HETE (10^{-7} M) after inhibition of the thromboxane-PGH_2 receptor with SQ-29548 (10^{-5} M; n=3) in the presence of indomethacin (10^{-5} M). (*p < 0.05 different from 20-HETE alone)

Figure 5. Percent constriction from baseline LD by 20-HETE (10^{-7} M) after inhibition of K_{Ca} channels with IBTX (10^{-7} M; n=6), CTX ($5X10^{-8}$ M; n=5) or apamin (10^{-6} M; n=5) in the presence of indomethacin (10^{-5} M). Inhibition of large, intermediate and small conductance K_{Ca}

channels significantly inhibited 20-HETE-induced constriction. (*$p < 0.05$ different from 20-HETE alone)

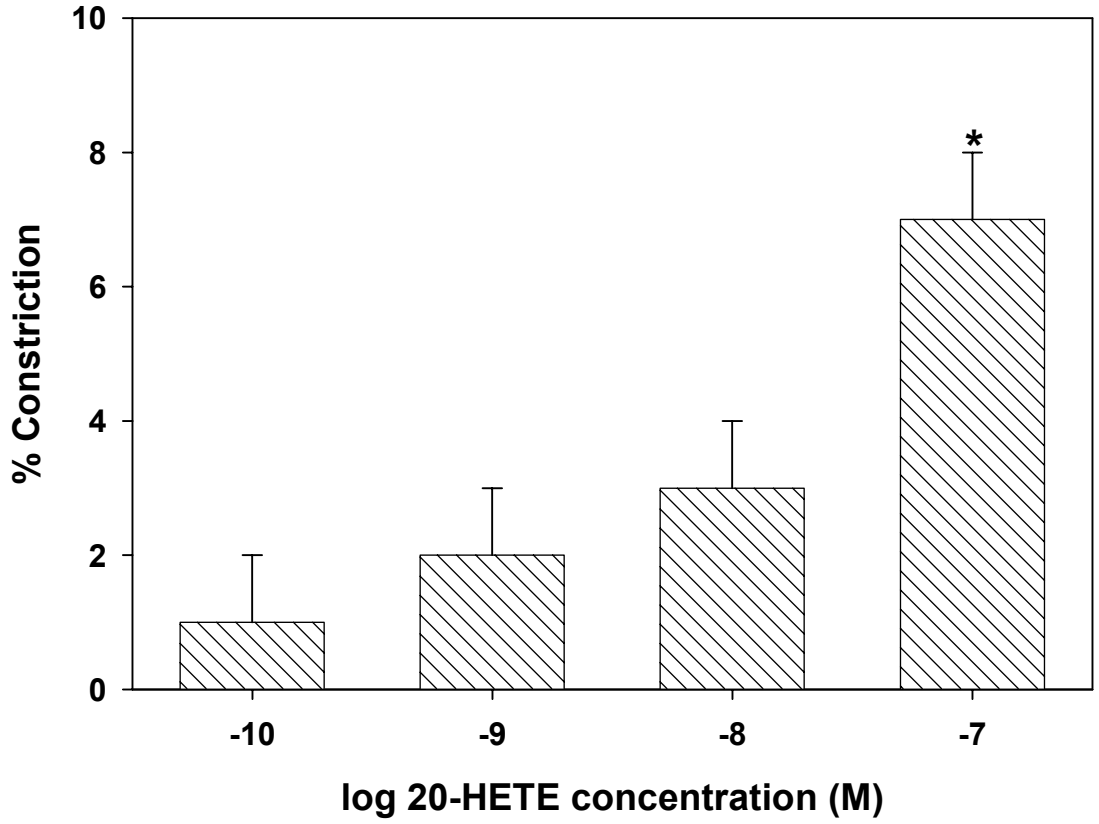


Figure 1

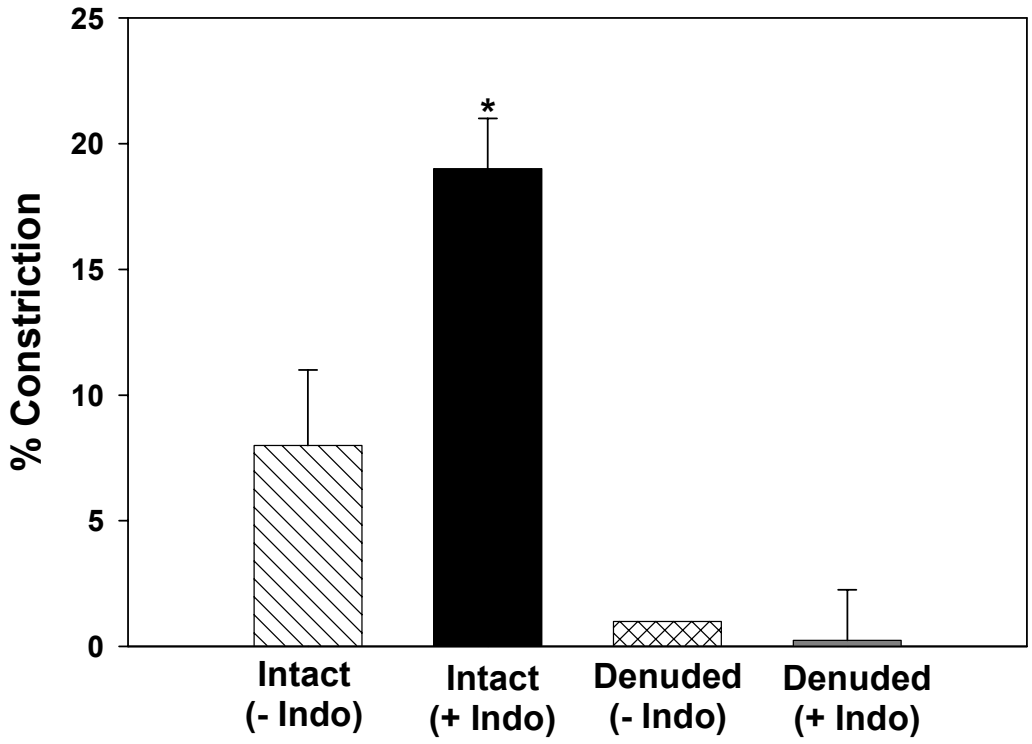

25
20
15
10
5
0
% Constriction
*
Intact
(- Indo)
Intact
(+ Indo)
Denuded
(- Indo)
Denuded
(+ Indo)

Figure 2

Figure 3A

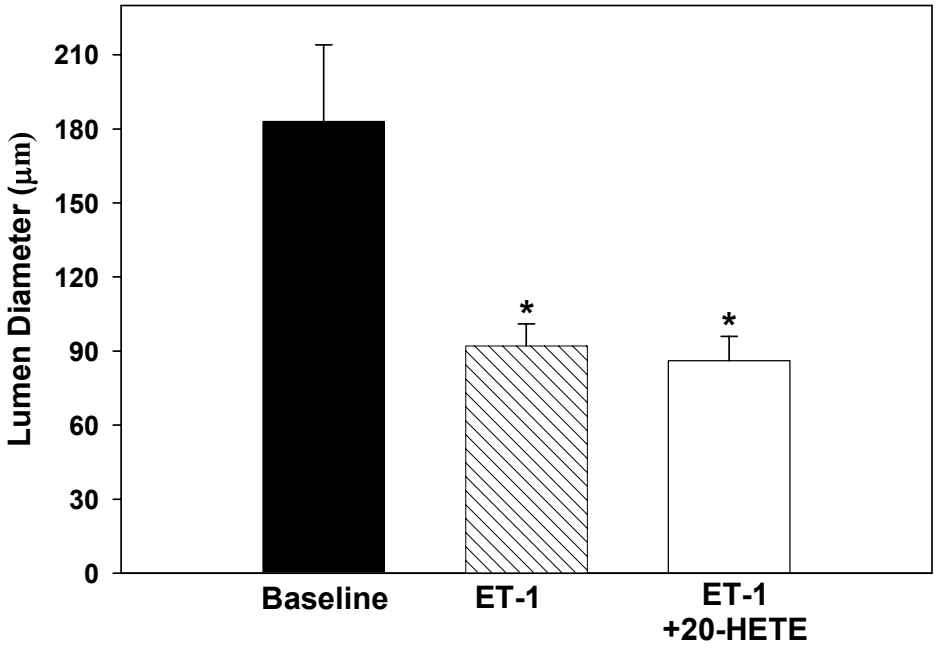
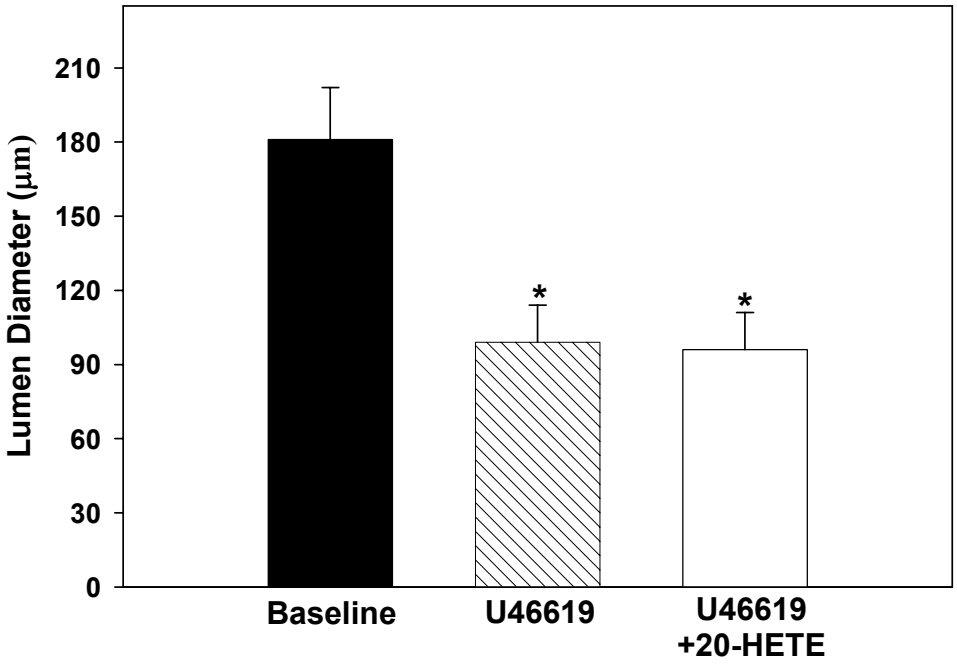


Figure 3B



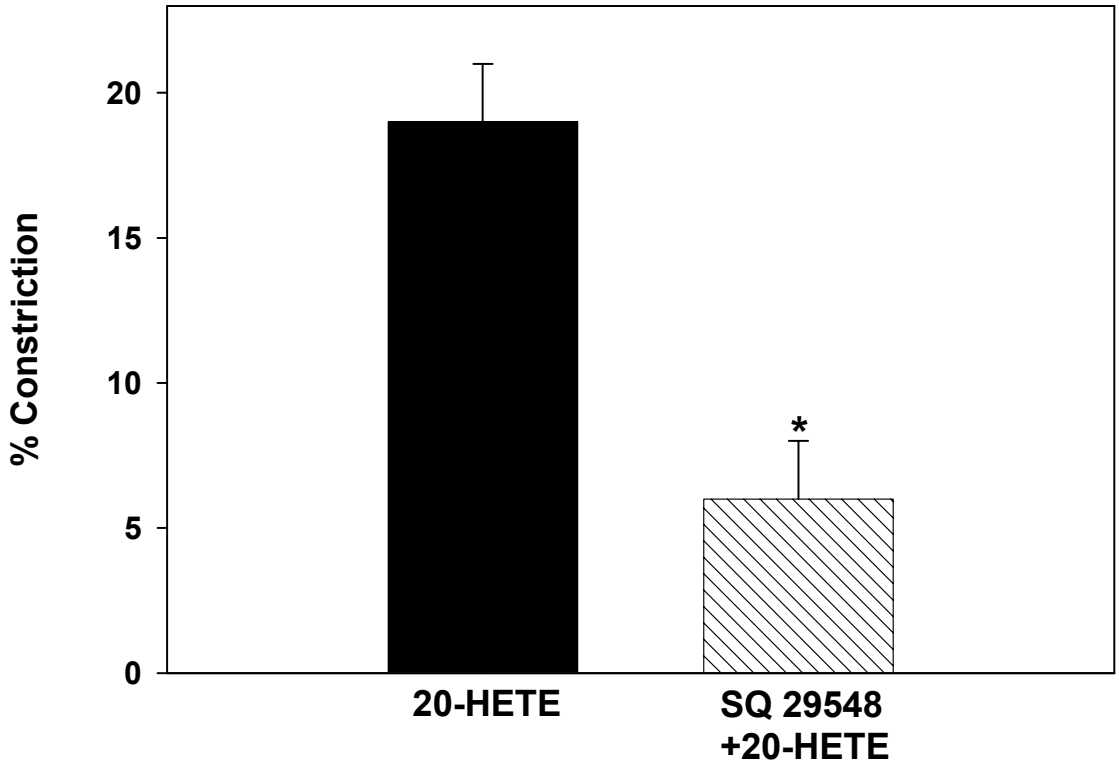


Figure 4

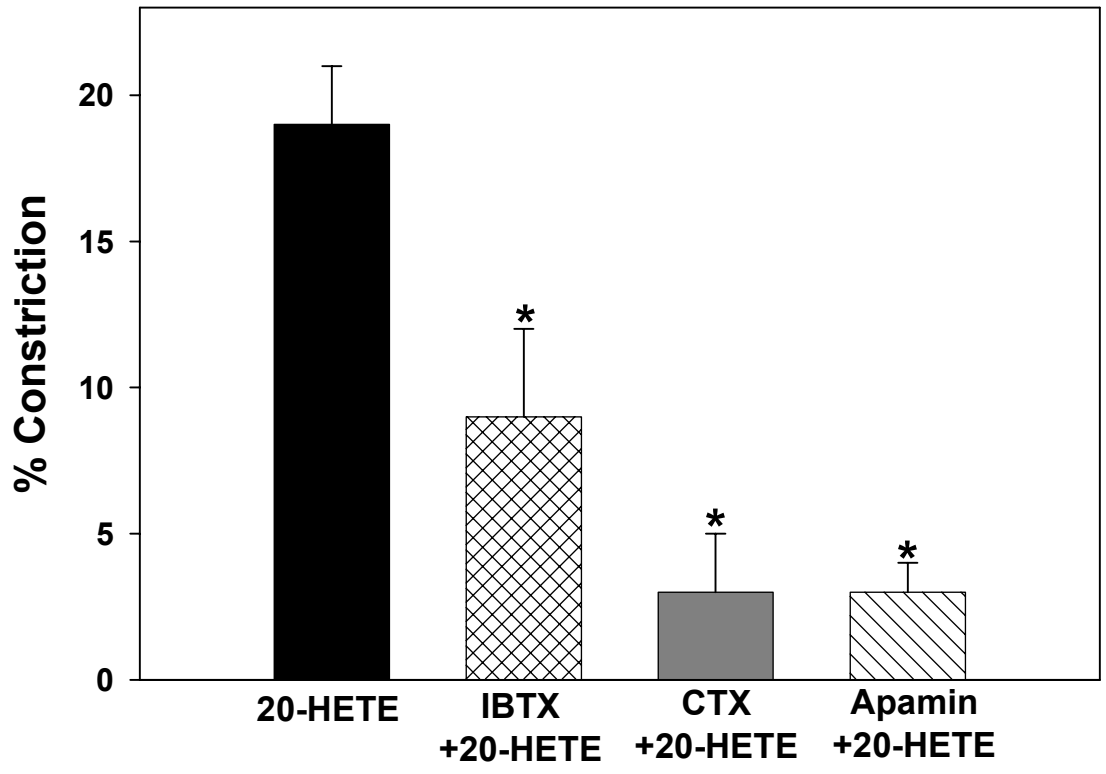

% Constriction
20
15
10
5
0
*
*
*
20-HETE
IBTX
+20-HETE
CTX
+20-HETE
Apamin
+20-HETE

Figure 5