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17-Octadecynoic acid improves contractile response to angiotensin II by releasing vasocontrictor prostaglandins

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ABSTRACT

The present study investigated the role of CYP-enzymes in the modulation of vasoconstrictor responses to angiotensin II in rabbit aortae. In arteries with the endothelium-intact (*E*+) the CYP-inhibitor, 17-octadecynoic acid (17 ODYA), increased the efficacy to angiotensin II (17-ODYA-effect) as well as simultaneous incubation with miconazole (epoxygenase-inhibitor) and CAY 10434 (ω-hydroxylase-inhibitor). The removal of endothelium (*E*−) caused potentiation of the 17 ODYA-effect. Therefore, endothelium-dependent and -independent mechanisms would be involved. 17-ODYA and miconazole reduced Ach-relaxation. Indomethacin blocked the 17-ODYA-effect in *E*+ and *E*− arteries but blunted the response to angiotensin II only in *E*+ arteries. NS 398 (cyclooxygenase-2-inhibitor) blocked the 17-ODYA-effect and reduced angiotensin II affinity as well as SQ 29548 (thromboxane-prostanoid (TP) receptor-inhibitor). In *E*− arteries, CAY 10434 enhanced angiotensin II response as well as 17-ODYA. SC 560 (cyclooxygenase-1-inhibitor) and NS 398 partially blocked the 17-ODYA-effect. In conclusion, 17-ODYA induced endothelial dysfunction by inhibiting CYP-epoxygenase and thus improves vasoconstrictor cyclooxygenase-2 metabolites release acting through TP receptors. The endothelium-independent mechanism of 17-ODYA-effect may involve increase of vasoconstrictor cyclooxygenase-metabolites induced by prostaglandin-ω-hydroxylase-inhibition.

1. Introduction

The functional relevance of vascular cytochrome P_{450} (CYP) enzymes has only been appreciated in the last 15–20 years; thus, the pathophysiological role of CYP-derived metabolites in the regulation of vascular homeostasis has not yet been fully elucidated. There is strong evidence suggesting that the activation of a CYP epoxygenase in endothelial cells is an essential step in NO and prostacyclin independent vasodilatation of several vascular beds. A smooth muscle CYP ω-hydroxylase, on the other hand, generates a vasoconstrictor eicosanoid. Evidence in recent years suggests a link between CYP expression/activity and cardiovascular disease. CYP pathways are altered in animal models of hypertension and atherosclerosis [1].

The arachidonic acid metabolizing CYP enzymes with prominent roles in vascular regulation are the epoxygenases of the 2 gene family (e.g., CYP 2B, 2C8, 2C9, 2C10, 2J2 in humans; 2C34 in pigs; 2C11, 2C23 and 2J4 in rats) which generate a series of regiospecific and stereospecific epoxides: 5,6-, 8,9-, 11,12-, and 14,15-epoxyeicosatrienoic acids (EETs), and the arachidonic acid ω-hydroxylases belonging to the CYP 4A family which form subterminal and ω-terminal hydroxyeicosatetraenoic acids (HETEs) [2]. Irizar and Ioannides [3] demonstrated the expression of CYP proteins belonging to families CYP2, CYP3 and CYP4 in rabbit aorta.

Accumulated evidence suggests that EDHFs are EETs [4]. These substances hyperpolarize endothelial and smooth muscle cells by activating calcium-dependent *K* channels (K_{Ca}) [2]. Imig et al. [5] demonstrated that inhibition of the CYP epoxygenase pathway greatly attenuated the nitric oxide-independent component of afferent arteriolar vasodilation in response to bradykinin. Moreover, EETs are vasodilatory, largely due to their ability to activate endothelial NO-synthase (eNOS) and NO-release [6,7].

Endothelial cells also can induce contractions of the underlying vascular smooth muscle by generating endothelium-dependent contracting factors (EDCFs). The endothelial COX-1 isoform of cyclooxygenase (COX) transforms arachidonic acid into endoperoxides which *per se* cause contraction of vascular smooth muscle [8,9]. Endoperoxides are converted further into prostacyclin, thromboxane A2, prostaglandin D2, prostaglandin E2 and/or prostaglandin F2α by their respective synthases. Of those enzymes, the prostacyclin synthase gene is by far the most abundantly expressed in

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endothelial cells, and more so in the SHR than in the WKY endothelium [10,11].

Vasoconstrictor prostanoids act on vascular smooth muscle cells via the thromboxane-prostanoid (TP) receptor [12]. Stimulation of TP receptors increases Ca^{2+} entry by opening of both voltage- and receptor-operated channels, with subsequent increase in cytosolic Ca^{2+} and smooth muscle contraction. Thus, any reduced endothelium-dependent relaxation, or even vasoconstriction, could result from either loss of EDRFs, (enhanced) generation of EDCFs, or a combination of both. EDCF-mediated vasoconstriction might prevail in the event of disturbed endothelial function such as in hypertension or hypercholesterolemia.

20-Hydroxyeicosatetraenoic acid (20-HETE), an ω-hydroxylation product of arachidonic acid catalyzed by CYP hydroxylase (CYP4A), is endogenously produced by smooth muscle after an increase in $[Ca^{2+}]_i$. 20-HETE increases smooth muscle tone by inhibiting large-K_{Ca}, inducing depolarization and directly effecting L-type Ca^{2+} channels [13]. Escalante et al. [14] demonstrated that 20-HETE is an endothelium-dependent vasoconstrictor in rabbit arteries.

Angiotensin II stimulates the synthesis and release of different endothelium dependent and independent factors: NO, free radicals and eicosanoids. Eicosanoids-release is primarily the consequence of phospholipase activation with attendant elevation in the amount of free arachidonic acid available for metabolism by COXs, lypoxygenases or CYP oxygenases [15]. Jerez et al. [16] demonstrated that angiotensin II contractile response is modulated by NO-release. Thus, in experimental conditions of endothelial dysfunction induced by NO shyntase inhibition, angiotensin II stimulated the endothelium dependent release of two COX-dependent eicosanoids one of which is also CYP-dependent [17]. The other acts through TP receptors and diminishes K_{Ca} activity.

Since relatively little is known about the role of CYP-enzymes metabolites in angiotensin II contractile response, and since there is evidence that angiotensin II stimulates the formation of 20-HETE in vascular smooth muscle [18], we have investigated the role of CYP-enzymes in the modulation of vasoconstrictor responses to angiotensin II in rabbit aortae.

2. Materials and methods

2.1. Rabbit aortic ring preparation

Experiments were performed on isolated rabbit thoracic aortae from male Flanders hybrid rabbits (1.5–2.5 kg). The rabbits were obtained from a slaughterhouse. Each thoracic aorta was carefully dissected and all adherent fat and connective tissue was removed. Five-millimeter-wide rings were cut and mounted in a 10 ml organ bath containing a Krebs solution composed of the following (mM): 128 NaCl, 4.7 KCl, 14.4 $NaHCO_3$, 1.2 NaH_2PO_4, 0.1 Na_2–EDTA, 2.5 $CaCl_2$, 1.1 glucose, pH 7.2. The Krebs solution was kept at 37 °C and aerated with 95% O_2 and 5% CO_2.

Isometric contractions were measured by using force–displacement transducers and were recorded under an initial tension of 2 g, which was found to be the optimal tension for KCl-induced contractions (100 mM). All preparations were allowed to equilibrate for 90 min and were washed with the Krebs solution at 15-min intervals. The endothelium was kept intact in one group of rings, but in other groups, the endothelium was removed by rubbing the luminal surface.

All experimental procedures complied with the Guide for the Care and Use of Laboratory Animals by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and were approved by our Institutional Committee for the Ethical Care of Animals.

2.2. Experimental design

2.2.1. Effects of CYP-inhibition on angiotensin II-contractile response

Endothelium-intact and endothelium-removed aortic rings were exposed to increasing doses of angiotensin II (10^{-10} to 2.5×10^{-6} M) to construct one cumulative concentration–response curve (CRC) in the absence (control) or presence of CYP-oxygenases inhibitors.

In one group, 17-octadecynoic acid (17-ODYA) 10^{-6} M was added to the bath 30 min before the angiotensin II-stimulation. Considering that Miyata and Roman [18] reported that 17-ODYA is not a specific inhibitor of the formation of 20-HETE by hydroxylases, and it is equally effective at inhibiting the formation of EETs by epoxygenases, the effect of more specific inhibitors was examined in another groups. Aortic rings were incubated either with miconazole 10^{-6} M (an inhibitor of CYP epoxygenase) or with CAY 10434×10^{-6} M (a specific inhibitor of CYP4A hydroxylase) or with miconazole plus CAY 10434. All inhibitors were added to the bath 30 min before the angiotensin II-stimulation.

2.2.2. Effects of CYP-inhibition on KCl and noradrenaline contractile response

Endothelium-intact and endothelium-removed aortic rings were separated into two groups. One group was exposed to KCl 100 mM. The other group was exposed to increasing doses of noradrenaline (10^{-8} to 10^{-3} M) to construct one CRC. Both protocols were addressed in the absence (control) or presence of 17-ODYA 10^{-6} M.

2.2.3. Relationship between NO and CYP

17-ODYA increased the maximal response to angiotensin II (17-ODYA-effect). Considering the relationship between CYP activity and NO-release [4,5], the role of NO in this phenomenon was evaluated. Endothelium-intact aortic rings were precontracted with a submaximal concentration of noradrenaline (5×10^{-6} M). As the plateau of the contraction is obtained, acetylcholine (Ach, 10^{-9} to 5×10^{-6} M) was added cumulatively. Ach was added such that the effect of the previous concentration had become maximal before the next one was added. 17-ODYA 10^{-6} M or miconazole 10^{-6} M were added to the bath 30 min before noradrenaline stimulation in absence (Control) or presence of the NO-synthase inhibitor N^G-nitro L-arginine methyl ester (L-NAME) 10^{-4} M. In other group, arteries were incubated with CAY 10434×10^{-6} M 30 min before noradrenaline stimulation in absence (control) or presence of miconazole.

17-ODYA and miconazole reduced Ach relaxation in endothelium-intact arteries. To study the endothelium specificity of this phenomenon, aortic rings were incubated with 17-ODYA or miconazole and were contracted with noradrenaline. As the plateau of the contraction was reached, arteries were exposed to increasing doses of sodium nitroprusside (NO-independent vasodilator).

The relaxation was expressed as percent change from preexisting (before addition of vasorelaxant) tone.

On the other hand, the effect of NO-synthase inhibition on the increase of the contractile response to angiotensin II induced by 17-ODYA was tested in endothelium-intact aortic rings. Arteries were incubated with L-NAME 10^{-4} M or L-NAME plus 17-ODYA.

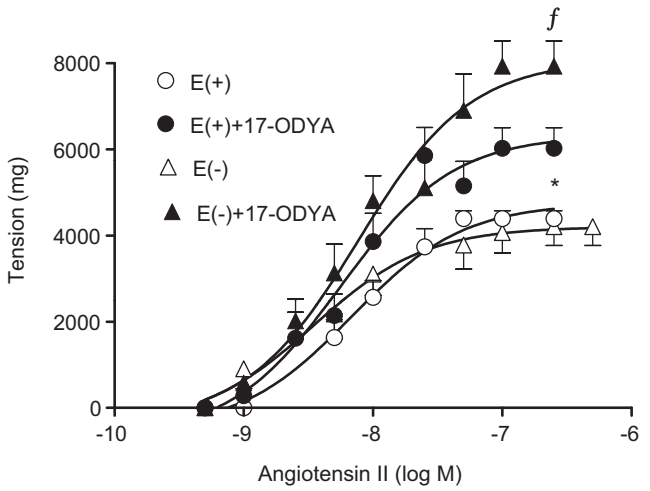


Fig. 1. Cumulative dose response curves to angiotensin II in aortic rings. *E*(+): endothelium-intact arteries; *E*(−): endothelium-removed arteries. *E*(+)+17-ODYA endothelium-intact arteries incubated with 17-ODYA 10^{-6} M; *E*(−)+17-ODYA endothelium-removed arteries incubated with 17-ODYA 10^{-6} M. Data are expressed as means ± S.E.M of 10 experiments.*$p < 0.01$ indicates statistically significant differences between untreated arteries and endothelium-intact arteries or endothelium-removed arteries incubated with 17-ODYA. *f* $p < 0.01$ indicates statistically significant differences between endothelium-intact arteries and endothelium-removed arteries treated with 17-ODYA (ANOVA and Duncan's test).

2.2.4. Role of COXs in the increase of the maximal response to angiotensin II induced by 17-ODYA

The role of COXs in 17-ODYA-effect was assessed. Endothelium-intact arteries and endothelium-removed arteries were incubated with indomethacin 10^{-5} M (COX-1 and COX-2 inhibitor), NS 398×10^{-7} M (COX-2 inhibitor) or SC 560×10^{-6} M (COX-1 inhibitor) in the presence or absence (control) of 17-ODYA 10^{-6} M. In endothelium-intact arteries, a similar protocol was performed by using SQ 29548×10^{-6} M (TP receptors blocker).

All drugs used in the determination of isometric contraction were added to the bath 30 min before the CRC to angiotensin II.

The results are expressed as mg of isometric contraction.

2.3. Statistical analysis

Data are presented as mean values ± SEM. The pD_2 (negative log of molar concentration of angiotensin II inducing 50% of the maximal contraction), and the maximal contractile response (E_{max}) were calculated using a curve-fitting analysis program (Graph Pad Software; San Diego, CA, USA). The significance of the differences between and within the groups was examined with an analysis of variance (ANOVA) for repeated measures followed by a Duncan's test.

3. Results

3.1. Effects of CYP-inhibition on angiotensin II-induced vasoconstriction

3.1.1. Effects of 17-ODYA

The contractile response to angiotensin II (10^{-9} to 2.5×10^{-6} M) was concentration-dependent. There were not significant differences in E_{max} or pD_2 values between endothelium-intact arteries and endothelium-removed arteries (Fig. 1).

Incubating segments of endothelium-intact and endothelium-removed arteries with 17 ODYA 10^{-6} M increased the E_{max} to angiotensin II (Fig. 1). In addition, rubbing off endothelium from arteries improved the E_{max} to angiotensin II with respect to endothelium-intact arteries.

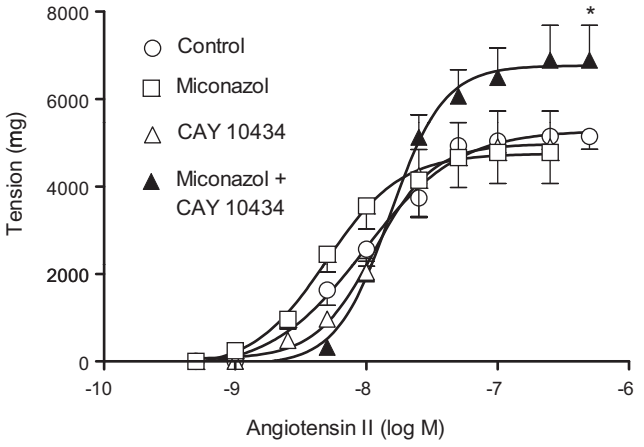


Fig. 2. Cumulative dose response curves to angiotensin II in endothelium-intact aortic rings. *$p < 0.01$ indicates statistically significant differences between arteries incubated with miconazole plus CAY 10434 and control (untreated) arteries and arteries treated with miconazole or CAY 10434 (ANOVA and Duncan's test).

3.1.2. Effects of miconazole and CAY 10434

Treatment of endothelium-intact arteries with miconazole 10^{-6} M or CAY 10434×10^{-6} M did not modify the E_{max} to angiotensin II with respect to the controls (Fig. 2). Miconazole 10^{-6} M ($pD_2 = 8.35 \pm 0.03$) improved angiotensin II affinity (pD_2 control = 8.18±0.08; $n = 10$, $p < 0.05$, ANOVA). Incubation of arteries with miconazole 10^{-6} M plus CAY 10434×10^{-6} M induced an increase in the E_{max} to angiotensin II of equal magnitude that the 17-ODYA 10^{-6} M (Fig. 2).

Contractile response to angiotensin II in endothelium-removed arteries was improved by CAY 10434×10^{-6} M as well as 17-ODYA 10^{-6} M (Table 1).

3.2. Effects of CYP-inhibition on KCl and noradrenaline contractile response

Contractile response to KCl 100 mM was similar in endothelium intact (3475 ± 845 mg) or endothelium removed arteries (3368 ± 692 mg). 17-ODYA did not modify contractile response to KCl either in arteries with endothelium (3836 ± 475 mg) or arteries with the endothelium disrupted (4596 ± 287 mg).

Contractile response to noradrenaline was slightly higher in endothelium intact aortic rings. However, the difference was not statistically significant (E_{max} endothelium-intact: 10,885 ± 1053 mg vs. E_{max} endothelium-removed: 8952 ± 600 mg;

Table 1
Values of maximal contractile response (E_{max}) and pD_2 to angiotensin II from endothelium-removed arteries.

	E_{max} (mg)	pD_2 (log M)
Control	5010 ± 307	8.26 ± 0.11
17-ODYA	7318 ± 618[a]	8.30 ± 0.07
Indomethacin	5221 ± 399	8.22 ± 0.12
17-ODYA + Indom	5133 ± 621[b]	8.00 ± 0.06
NS 398	5243 ± 630	7.74 ± 0.09[c]
17-ODYA + NS 398	6318 ± 409	7.92 ± 0.06
SC 560	5534 ± 364	8.00 ± 0.14
SC 560 + 17-ODYA	6236 ± 442	7.97 ± 0.16
CAY 10434	6764 ± 602[a]	7.86 ± 0.30

[a] $p < 0.05$ indicates statistically significant differences between arteries treated with 17-ODYA or CAY 10434 and untreated arteries (control).

[b] $p < 0.05$ indicates statistically significant differences between arteries incubated with 17-ODYA and incubated with 17-ODYA plus indomethacin (ANOVA and Duncan's test).

[c] $p < 0.05$ indicates statistically significant differences between arteries incubated with NS 398 and untreated arteries (control) (ANOVA and Duncan's test).

$n = 10$, $p > 0.05$). Incubation with 17-ODYA reduced affinity of noradrenaline in endothelium-intact (pD_2 control: 6.36 ± 0.06 vs. pD_2 17-ODYA: 5.43 ± 0.14; $n = 10$, $p < 0.05$) and endothelium-removed (pD_2 control: 6.43 ± 0.05 vs. pD_2 17-ODYA: 5.80 ± 0.19; $n = 10$, $p < 0.05$) aortic rings. The contractile response to noradrenaline was lower in arteries with endothelium incubated with 17-ODYA than in arteries untreated (E_{max} 7499 ± 621 mg; $n = 10$, $p < 0.05$, ANOVA and Duncan's test). In endothelium-removed arteries, 17-ODYA had no effect on maximal contractile response (E_{max} 9350 ± 870 mg; $n = 10$, $p > 0.05$, ANOVA and Duncan's test).

3.3. Relationship between NO and CYP

3.3.1. Response to acetylcholine

L-NAME 10^{-4} M reduced but not abolished Ach-maximal relaxation (E_{max} control: $71.7 \pm 7.7\%$; E_{max} L-NAME: $15.7 \pm 4\%$). 17-ODYA 10^{-6} M and miconazole 10^{-6} M significantly reduced Ach-maximal relaxation (E_{max} 17-ODYA: $37.7 \pm 3.9\%$; E_{max} miconazole: $33.4 \pm 7.0\%$; $n = 8$, $p < 0.05$, two way ANOVA and Duncan's test). In arteries incubated with L-NAME 10^{-4} M, 17-ODYA and miconazole blocked the ACh-relaxation.

CAY 10434×10^{-6} M has not effect on Ach-response (E_{max} CAY 10434: $68.4 \pm 6.3\%$) and did not restore Ach-relaxation in arteries incubated with miconazole 10^{-6} M (E_{max} miconazole plus CAY 10434: $35.3 \pm 8.8\%$).

3.3.2. Response to sodium nitroprusside

Maximal relaxation to sodium nitroprusside was reached at 10^{-6} M and was similar in arteries with endothelium ($81 \pm 8\%$) and arteries endothelium-removed ($89 \pm 2\%$). 17-ODYA did not change sodium nitroprusside-relaxation either in aortic rings with endothelium ($76 \pm 3\%$) or endothelium-removed ($85 \pm 6\%$; $n = 8$, $p > 0.05$, ANOVA and Duncan's test).

3.3.3. Effects of L-NAME in the contractile response to angiotensin II

L-NAME 10^{-4} M increased E_{max} to angiotensin II in endothelium intact arteries (E_{max} untreated: 4454 ± 266 mg vs. E_{max} L-NAME: 6086 ± 1044 mg; $n = 8$, $p < 0.05$). The maximal response to angiotensin II in endothelium-intact arteries incubated with L-NAME 10^{-4} M plus 17-ODYA 10^{-6} M was greater than the maximal response to angiotensin II in arteries treated with 17-ODYA 10^{-6} M (E_{max} 17-ODYA: 6025 ± 479 mg; E_{max} 17-ODYA plus L-NAME: 7386 ± 1146 mg; $n = 8$, $p < 0.05$). The magnitude of the increase was similar to endothelium-removed arteries incubated with 17-ODYA (E_{max}: 7318 ± 618 mg; $n = 8$, ns, ANOVA and Duncan's test).

3.4. Role of COXs in the increase of the maximal response to angiotensin II induced by 17-ODYA

3.4.1. Effects of indomethacin

Incubation of endothelium-intact aortic rings with indomethacin 10^{-5} M did not modify either the contractile response or the affinity to angiotensin II (Fig. 3a). However, in arteries treated with indomethacin 10^{-5} M plus 17-ODYA 10^{-6} M, indomethacin 10^{-5} M not only blocked the increase in the E_{max} induced by 17-ODYA10^{-6} M but also blunted the response to angiotensin II with respect to the control. In addition, a significant shift to the right of the CRC with respect to control arteries and those treated either with 17-ODYA 10^{-6} M or indomethacin 10^{-5} M was observed ($pD_2$17-ODYA plus indomethacin = 7.78 ± 0.06 vs. pD_2 control = 8.18 ± 0.12 or $pD_2$17-ODYA = 8.14 ± 0.07 or pD_2 indomethacin = 8.26 ± 0.05; $n = 10$, $p < 0.01$, one way ANOVA).

Incubation of endothelium-removed arteries with indomethacin 10^{-5} M did not modify either E_{max} or affinity to angiotensin II with respect to the controls. However, incubation of endothelium-removed arteries with indomethacin 10^{-5} M plus 17-ODYA blocked the increase in the E_{max} to angiotensin II induced by 17-ODYA and shifted the CRC to the right (Table 1).

3.4.2. Effects of NS 398, SC 560 and SQ 29548

Treatment of endothelium-intact arteries with NS 398×10^{-7} M or SQ 29548×10^{-6} M did not modify the contractile response or the affinity to angiotensin II with respect to the controls (Fig. 3b and d). SC 560×10^{-6} M reduced the contractile response to angiotensin II with respect to the untreated arteries (E_{max} SC 560 = 3710 ± 318 mg; E_{max} control: 5114 ± 264 mg; $n = 8$, $p < 0.05$, unpaired t-test). NS 398×10^{-7} M and SC 560×10^{-6} M blocked the increase in the E_{max} to angiotensin II induced by 17-ODYA 10^{-6} M. NS 398×10^{-7} M shifted the CRC to the right, but SC 560×10^{-6} M did not shift the CRC to the right (Fig. 3b and c). In turn, SQ 29548×10^{-6} M did not block the increase in the E_{max} to angiotensin II induced by 17-ODYA 10^{-6} M, but SQ 29548×10^{-6} M shifted the CRC to the right (Fig. 3d).

In endothelium-removed arteries, neither SC 560×10^{-6} M nor NS 398×10^{-7} M modified E_{max} with respect to the controls. SC 560×10^{-6} M and NS 398×10^{-7} M were capable of partially blocking the increase in E_{max} induced by 17-ODYA 10^{-6} M (Table 1). In turn, NS 398×10^{-7} M shifted the CRC of angiotensin II to the right with respect to the controls (Table 1).

4. Discussion

In this study, we have found that simultaneous inhibition of CYP-enzymes with 17-ODYA increased the efficacy to angiotensin II in endothelium-intact and endothelium-removed arteries. In contrast, 17-ODYA reduced the contractile response of noradrenaline in endothelium-intact and did not change the contractile response to KCl in endothelium-intact or endothelium-removed aortic rings. Furthermore, the effect of 17-ODYA was specific to the contractile response to angiotensin II. Removal of endothelium caused greater potentiation of the contractile response to angiotensin II in presence of 17-ODYA. 17-ODYA also reduced Ach-response in endothelium-intact arteries and did not modify sodium nitroprusside-relaxation either in endothelium-intact or endothelium-removed arteries. These results imply that endothelial dysfunction induced by 17-ODYA may account for the increase in the contractile response to angiotensin II; however, there are also endothelium-independent mechanisms involved in the 17-ODYA-effect.

17-ODYA is equally effective at inhibiting the formation of EETs as it is at inhibiting the formation of 20-HETE [18,19]. Campbell et al. [20] demonstrated that NO is not the only mediator of Ach-induced relaxations in the rabbit aorta. According to these authors, we found that L-NAME greatly reduced but did not abolish the Ach-relaxation. Even 17-ODYA and miconazole blocked the Ach-relaxation in arteries incubated with L-NAME. Taken these data into account, we tested the hypothesis that endothelium-dependent mechanisms of the 17-ODYA-effect on the angiotensin II response would involve dysfunction induced by epoxygenase inhibition. As stated in the introduction, EETs activate eNOS and NO-release [6,7]. Thus, the effect of specific epoxygenase inhibition in the endothelium-dependent effect of 17-ODYA was evaluated. In agreement with Campbell et al. [20] we found that miconazole reduced Ach-induced relaxations. However, miconazole did not alter the E_{max} to angiotensin II. This result indicates that the inhibition of arachidonic acid-epoxidation may induce endothelial dysfunction but there is an additional mechanism involved in the increase of the E_{max} to angiotensin II induced by 17-ODYA.

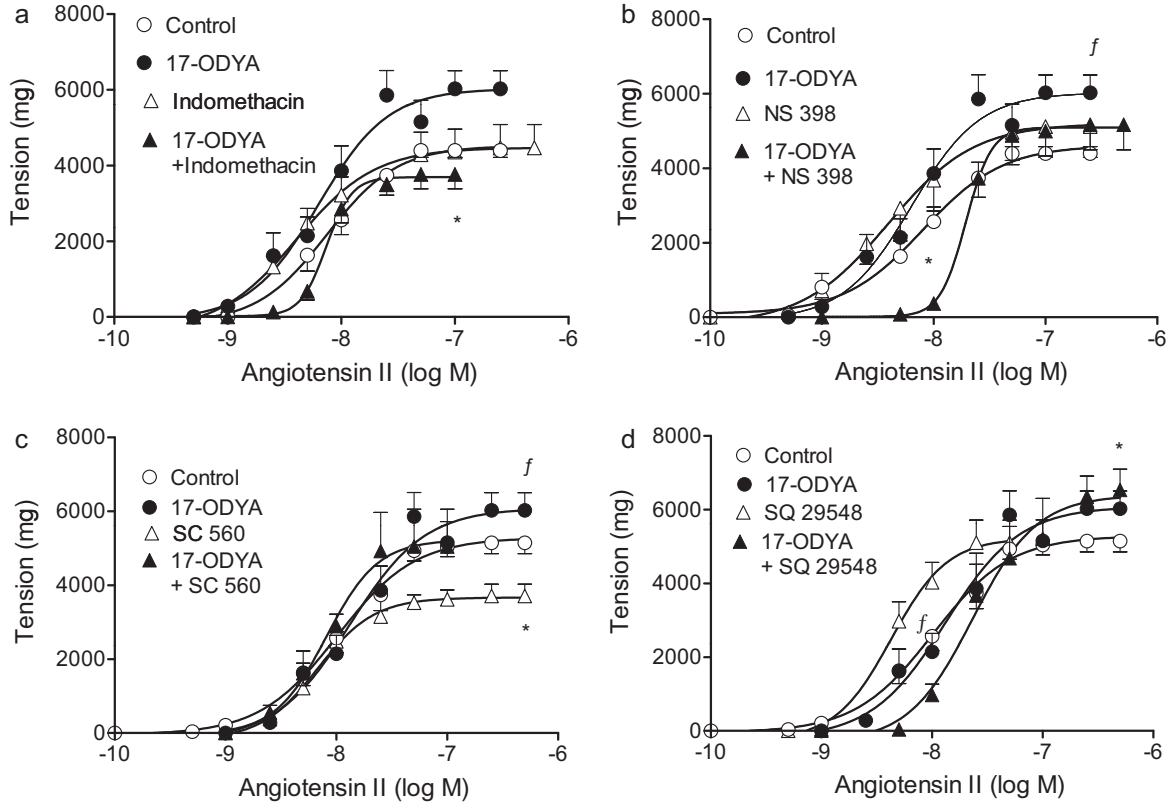
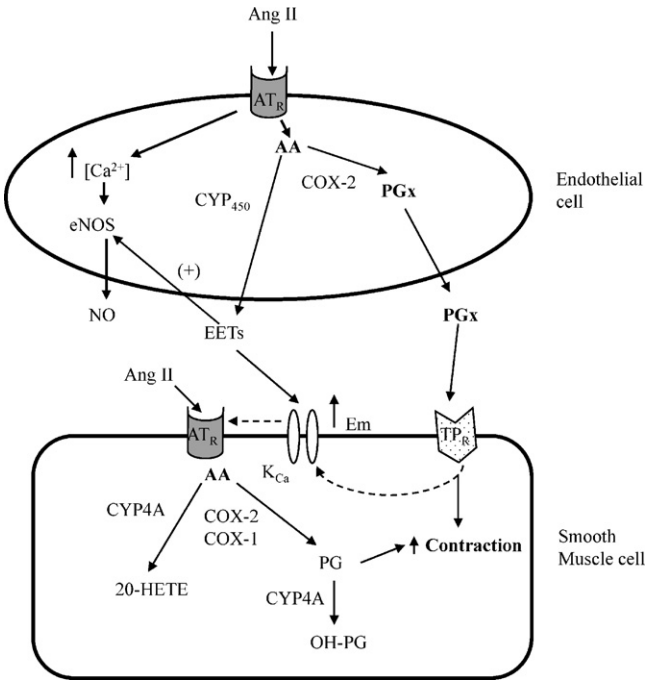


Fig. 3. Cumulative dose response curves to angiotensin II in endothelium-intact aortic rings. (a) Arteries were incubated with 17-ODYA 10^{-6} M, indomethacin 10^{-5} M or 17-ODYA plus indomethacin. *$p < 0.01$ indicates statistically significant differences between arteries incubated with 17-ODYA plus indomethacin and control (untreated) arteries and arteries treated with 17-ODYA or indomethacin (ANOVA and Duncan's test). (b) Arteries were incubated with 17-ODYA 10^{-6} M, NS 398 $\times$ 10^{-7} M or 17-ODYA plus NS 398. *$p < 0.01$ indicates statistically significant differences between pD_2 from arteries incubated with 17-ODYA plus NS 398 and control (untreated) arteries and arteries treated with NS 398 (ANOVA and Duncan's test). $fp < 0.01$ indicates statistically significant differences between E_{max} from arteries incubated with 17-ODYA and control (untreated) arteries and arteries treated with NS 398 and arteries treated with 17-ODYA plus NS 398 (ANOVA and Duncan's test). (c) Arteries were incubated with 17-ODYA 10^{-6} M, SC 560 $\times$ 10^{-6} M or SC 560 plus 17-ODYA. *$p < 0.01$ indicates statistically significant differences between E_{max} from arteries incubated with SC 560 and control (untreated) arteries (ANOVA and Duncan's test). $fp < 0.01$ indicates statistically significant differences between E_{max} from arteries incubated with 17-ODYA and control (untreated) arteries and arteries treated with SC 560 and arteries treated with 17-ODYA plus SC 560 (ANOVA and Duncan's test). (d) Arteries were incubated with 17-ODYA 10^{-6} M, SQ 299548 10^{-6} M or 17-ODYA plus SQ 29548. *$p < 0.01$ indicates statistically significant differences between E_{max} from arteries incubated with 17-ODYA or 17-ODYA plus SQ 29548 and control (untreated) arteries and arteries treated with SQ 29548 (ANOVA and Duncan's test). $fp < 0.01$ indicates statistically significant differences between pD_2 from arteries incubated with 17-ODYA plus SQ 29548 and control (untreated) arteries and arteries treated with SQ 29548 and arteries treated with 17-ODYA (ANOVA and Duncan's test).

Next, the effect of specific ω-hydroxylase inhibition in the endothelium-dependent effect of 17-ODYA was evaluated. Inhibition of arachidonic acid-ω-hydroxylation by CAY 10434 did not modify either Ach relaxation or the angiotensin II response in endothelium-intact arteries. The principal ω-hydroxylase metabolite from arachidonic acid, 20-HETE, sensitizes smooth muscle to the vasocontrictor response [13,21] and 17-ODYA is an inhibitor of 20-HETE production. Angiotensin II stimulates the formation of 20-HETE in vascular smooth muscle [18,22]. In consequence, CAY 10434 must reduce the contractile response to angiotensin II. Actually, we found that CAY 10434 improved E_{max} to angiotensin II in endothelium-removed arteries. The present results would imply that ω-hydroxylase inhibition increases the release of vasoconstrictor metabolites from smooth muscle. Endothelium-intact arteries compensate for this effect by releasing NO. This hypothesis may be supported by the following findings: (1) Alonso Galicia et al. [23] found that inhibiting of the production of 20-HETE contributes to the vasodilatory effects of NO. (2) Angiotensin II increases NO-release from endothelium [16] and L-NAME improved the contractile response to angiotensin II in endothelium-intact aortic rings. (3) The contractile response to angiotensin II in arteries incubated with L-NAME plus 17-ODYA was similar to the response in endothelium-removed arteries incubated with 17-ODYA.

As stated previously, 17-ODYA increased the contractile response to angiotensin II in endothelium-intact arteries, but neither miconazole nor CAY 10434 increased the contractile response. Therefore, we tested the hypothesis that the 17-ODYA-effect on the angiotensin II response is a consequence of simultaneous inhibition of arachidonic acid-ω-hydroxylation and epoxidation. We found that incubation with miconazole plus CAY 10434 induced an increased efficiency to angiotensin II of equal magnitude that the 17-ODYA. Even, CAY 10434 did not restore the reduction in the Ach-relaxation induced by miconazole. These findings suggest that endothelium dysfunction induced by epoxygenase inhibition, in addition to increased vasoconstrictor metabolite-release induced by ω-hydroxylase inhibition, may account for the improvement of the contractile response to angiotensin II in the presence of 17-ODYA.

The CYP4A subfamily encodes several cytochrome P450 enzymes that are capable of hydroxylating the terminal omega-carbon and, to a lesser extent, the (omega-1) position of saturated and unsaturated fatty acids, as well as enzymes active in the omega-hydroxylation of various prostaglandins [24,25]. Granstrom [26] has studied prostaglandin omega-hydroxylation and shown that the prostaglandins are further metabolized to dicarboxilic acid. The omega-oxidation may be the first step of biological



Scheme 1. Representation of the relationship between the cytochrome P_{450} epoxygenase, the cytochrome P_{450}-ω-hydroxylase and metabolites from COX in the vascular response. to angiotensin II. Continuous lines represent activation. Discontinuous lines represent inhibition. Ang II: angiotensin II; AT_R: angiotensin II receptor; COX: cyclooxygenase; CYP_{450}: cytochrome P_{450} epoxygenase; CYP4A: cytochrome P_{450} ω-hydroxylase; EETs: epoxyeicosatrienoic acids; Em: membrane potential; eNOS: endothelial nitric oxide synthase; 20-HETE: 20-hydroxyepoxyeicosatetraenoic acid; AA: arachidonic acid; PG: prostaglandin; OH-PG: hydroxyprostaglandin; K_{Ca} calcium-activated potassium channels; TP: thromboxane A_2/prostaglandin H_2 receptor; PGx: COX-2 dependent prostaglandin released by angiotensin II in endothelium intact arteries incubated with 17-ODYA.

inactivation of prostaglandins, and therefore may play a role in the regulation of prostaglandin levels in smooth muscle [27,28]. Considering the effect of CAY 10434 in endothelium-removed arteries, we hypothesized that a prostaglandin omega hydroxylase metabolizes vasoconstrictor prostaglandins to inactive metabolites in rabbit aortic smooth muscle. The inhibition of the 17-ODYA-effect with indomethacin we found both in endothelium intact and endothelium-removed arteries is consistent with this hypothesis.

In endothelium-intact arteries, indomethacin not only blocked the 17-ODYA effect but also blunted the contractile response to angiotensin II. In previous study, Jerez et al. [17] demonstrated that in conditions of NOS-inhibition there is an increase of free radicals that activates COX to improve the synthesis of vasoconstrictor prostanoids. Taking into account that 17-ODYA reduced Ach-relaxation, there would be endothelium-dependent prostaglandins involved in the effect of the 17-ODYA on the contractile response to angiotensin II. SC 560 reduced the angiotensin II response in endothelium-intact but not in endothelium-removed arteries. In agreement with Qi et al. [29] this finding indicates that endothelium-dependent COX-1 metabolites play a role in the contractile response to angiotensin II in basal conditions. SC 560 blocked the increase in the contractile response to angiotensin II induced by 17-ODYA, but SC 560 did not blunt the contractile response. This result would mean that COX-2 vasoconstrictor metabolites are involved in the 17-ODYA effect. NS 398 restored the E_{max} and shifted the CRC to angiotensin II to the right in arteries incubated with 17-ODYA. Thus, COX-2 metabolites derived from endothelium together with endothelial dysfunction induced by epoxygenase inhibition may be involved in the increased efficiency of angiotensin II-contractile response induced by 17-ODYA. According to that, Heymes et al. [30] and Shi et al. [31] reported that even though COX-1 is the major isoenzyme in vasculature, the two isoforms of COX contribute to augmented endothelium response contractions.

We assessed whether the COX metabolites released during 17-ODYA-incubation interact with a TP receptor. SQ 29548 did not block the increase in the contractile response to angiotensin II induced by 17-ODYA. However, SQ 29548 acted as a competitive inhibitor, causing parallel rightward shifts of the CRC to angiotensin II in such conditions. Thus, COX-2 metabolites released during 17-ODYA-incubation act through TP receptors and modulate angiotensin II affinity. Wong et al. [32] found that PGF_2, derived from COX-2, is the most likely EDCF underlying endothelium-dependent, thromboxane – prostanoid receptor – mediated contractions in hamster aortae.

In endothelium-removed arteries, indomethacin blocked the 17-ODYA-effect. Both NS 398 and SC 560 partially blocked the increase of the E_{max} to angiotensin II induced by 17-ODYA. Thus, endothelium-independent vasoconstrictor prostaglandins derived from either COX-1 or COX-2 would be increased during the prostaglandin omega hydroxylase inhibition by 17-ODYA.

According to the evidence obtained in this work, we can state that the factors responsible for the improvement of the response to angiotensin II during simultaneous inhibition of arachidonic acid epoxidation and ω-hydroxylation are the followings: (1) Endothelial dysfunction induced by epoxygenase inhibition. (2) The increase in the release of vasoconstrictor COX-2 metabolites from endothelium. (3) The improvement of vasoconstrictor endothelium-independent COXs-metabolites induced by prostaglandin hidroxylase inhibition (Scheme 1).

5. Conclusion

The results of the present investigations demonstrate a close relationship between CYP epoxygenase and ω-hydroxylase together with COX-metabolites in the contractile response to angiotensin II in rabbit aortic rings. The interactions between CYP enzymes involve both endothelium and smooth muscle functions. Taking into account that the pathophysiological role of CYP-derived metabolites has not yet been fully elucidated, the present work may constitute a significant contribution to explain the role of CYP-derived metabolites in the regulation of vascular homeostasis.

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