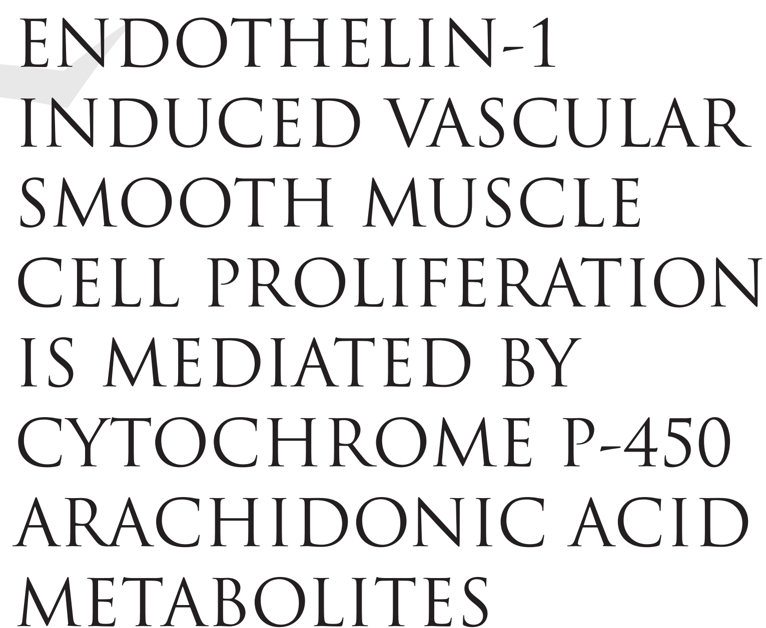

ENDOTHELIN-1 INDUCED VASCULAR SMOOTH MUSCLE CELL PROLIFERATION IS MEDIATED BY CYTOCHROME P-450 ARACHIDONIC ACID METABOLITES

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Abstract

Endothelins (ETs) are a family of three peptides (ET-1, ET-2, ET-3) that are implicated in the physiological control of vascular smooth muscle cell (VSMC) and myocardial contractility and growth. ET-1 is vasoactive peptide that acts via ET-A receptors coupling inducing vascular smooth muscle cell contraction. ET-1 is involved in the development and maintenance of hypertension.
Aim of this study was to investigate whether ET-1 can induce vascular smooth muscle cell proliferation through arachidonic acid (AA) metabolites formed via cytochrome P¬450 (CYP-450). VSMC proliferation was measured by [^{3}H]thymidine incorporation in cultured cells treated by ET-1 (10 to loo nmol/L) in presence of different inhibitors of CYP-450 (17-ODYA 5 μmol/L), lipoxygenase (LO) (baicalein 20 μmol/L) and cyclooxygenase (COX) (indomethacin 5 μmol/L). ET-1 (10 to 100 nmol/L) induced VSMC proliferation and this effect was attenuated by CYP-450 inhibitor (17-ODYA) and lipoxygenase (LO) inhibitor (baicalein) but not by cyclooxygenase (COX) inhibitor (indomethacin). CYP-450 and LO metabolites of AA, 20-hydroxyeicosatetraenoic acid (HETE) and 12-HETE increased [^{3}H]thymidine incorporation in VSMC. Inhibitors of MAP kinase (PD-98059 50 μmol/L) and cPLA2 (MAFP 50 μmol/L) attenuated ET-1 as well as 20-HETE induced VSMC proliferation. These results suggest AA metabolites via CYP-450 mediates ET-1 induce VSMC proliferation.

KEY WORDS: endothelin-1, vascular smooth muscle cell proliferation, cytochrome P-450, arachidonic acid

Introduction

Vasoactive agents such as norepinephrine (NE), angiotensin II (ANG II), and endothelin-1 can induce hypertrophy and proliferation of vascular smooth muscle cell and thus be implicated in pathogenesis of hypertension (1-5). Endothelins (ETs) are a family of three peptides (ET-1, ET-2, ET-3) that are implicated in the physiological control of vascular smooth muscle cell (VSMC) and myocardial contractility and growth. ET-1 is vasoactive peptide that acts via ET-A receptors coupling inducing vascular smooth muscle cell contraction (6-8). ET-1 induces arachidonic acid release via activation of cytosolic phospholipase A2 (cPLA2) (9). Arachidonic acid is metabolized by cyclooxygenase into prostaglandins, by lipoxygenase into leukotrienes and by cytochrome P-450 into 12-, 19-, and 20-HETE (10, 11). It has been reported that NE and ANG II induce arachidonic acid release via activation of CaMK II and cPLA2 (12, 13). The metabolites of arachidonic acid generated via cytochrome P-450 and lypoxygenase contribute to development of hypertension and vascular smooth muscle cell proliferation (12, 13, 14). Aim of this study was to investigate whether ET-1 can induce vascular smooth muscle cell proliferation through arachidonic acid (AA) metabolites formed via cytochrome P¬450.

Materials and Methods

Materials

Hanks' balanced salt solution, M-199, phosphate buffered saline, bovine serum albumin, dithiothreitol, EGTA, Endothelin-1, phenylmethylsul¬fonyl fluoride, soybean trypsin inhibitor, and myelin basic protein were purchased from Sigma. Leupeptin and aprotinin were from Calbiochem; [methyl-^{3}H]thymidine (20 Ci/mmol) was obtained from Du Pont-NEN; and [γ-^{32}P] ATP (3000 Ci/mmol) was obtained from Amersham. MEK inhibitor PD-98059 was obtained from New England Biolabs; cPLA2 inhibitor MAFP and 12(5)-, and 20-HETE were from Cayman Chemicals. COX inhibitor, Indomethcin, CYP-450 and LO inhibitors, 17-ODYA and Baicalein were purchased from Biomol. All inhibitors were dissolved in dimethyl sulfoxide and further diluted with M-199 for experiments.

Methods

Measurement of DNA Synthesis by [^{3}H]Thymidine Incorporation

Incorporation of [^{3}H]thymidine into DNA was measured in aortic smooth muscle cells. Subconfluent cells from fifth through ninth passages were incubated with 0,05% FBS containing M-199 for 4 hours to induce mitogenic quiescence. Cells were incubated with endothelin-1 (10 to 100 nmoI/L) or its vehicle for 48 hours and 0.5 μCi/mL [^{3}H]thymidine was added to the cultures in each well during the last 24 hours of incubation period. This time of incubation with ET-1 and [^{3}H]thymidine resulted in maximal [^{3}H]thymidine incorporation in VSMC. To investigate the contribution of AA metabolites to the action of ET-1 on DNA synthesis, quiescent cells were pre-incubated with IND BACL, 17-ODYA for 30 minutes or PD-98059 for 4 hours, inhibitors of COX, LO, and CYP-450 (5-50 μmol/L), respectively, or their vehicles and then exposed to ET-1 (10 do 100 nmol/L) for 4 hours in the presence of the above inhibitors. In all cells [^{3}H] thymidine incorpora¬tion was normalized for protein content and is expressed as counts per minute per well.

Statistical Analysis

Values are expressed as mean ± SEM. The data were analyzed by 1-way ANOVA, and difference between the means for multiple comparisons was determined by the Newman-Keuls test and a value difference of $p<0,05$ was considered statistically significant.

Results

To determine the effect of ET-1 on vascular smooth muscle cell proliferation aortic vascular smooth muscle cells made quiescent for 24 hours (M-199 arresting medium), were exposed to ET-1. ET-1 increased [^{3}H]thymidine incorporation in VSMC in a concentration dependent manner (Figure 1).

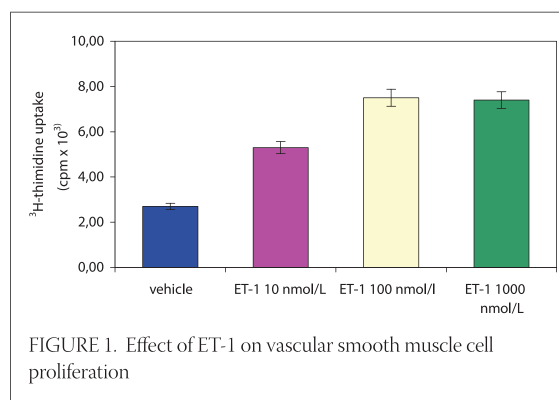

FIGURE 1. Effect of ET-1 on vascular smooth muscle cell proliferation

COX, LO and Citochrome P-450 monooxygenase metabolite arachidonic acid into prostaglandins, leukotrienes and 12-, 20-HETE, respectively. To determine whether activities of these enzymes and their metabolites are involved in ET-1 induced VSMC pro-

liferation, cells were pretreated by inhibitors of COX (indomethacin), LO (baicalein) and CYP-450 (17-ODYA) and than treated by ET-1 100 nmol/L. CYP-450 and LO inhibitors, but not COX inhibitor, attenuated [^{3}H]thymidine incorporation in VSMC (Figure 2).

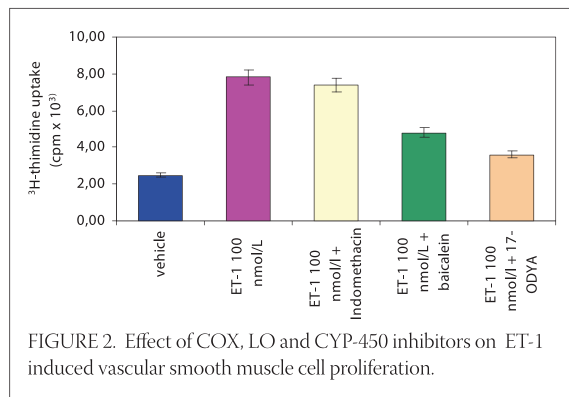

FIGURE 2. Effect of COX, LO and CYP-450 inhibitors on ET-1 induced vascular smooth muscle cell proliferation.

20-hydroxyeicosatetraenoic acid (HETE) increased [^{3}H]thymidine incorporation in VSMC. Inhibitors of MAP kinase (PD-98059 50 μmol/L) and cPLA2 (MAFP 50 μmol/L) attenuated ET-1 as well as 20-HETE induced VSMC proliferation (Figures 3. and 4.).

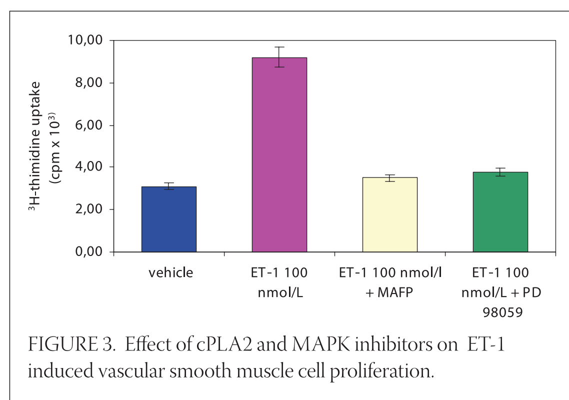

FIGURE 3. Effect of cPLA2 and MAPK inhibitors on ET-1 induced vascular smooth muscle cell proliferation.

Discussion

It has been previously reported that norepinephrine (NE) and angiotensin II (ANG II) increase CaM kinase II and cPLA2 activities and thus release arachidonic acid (12). The metabolites of arachidonic acid, such as 12- and 20-HETE made via cytocrome P-450 and lipoxy-

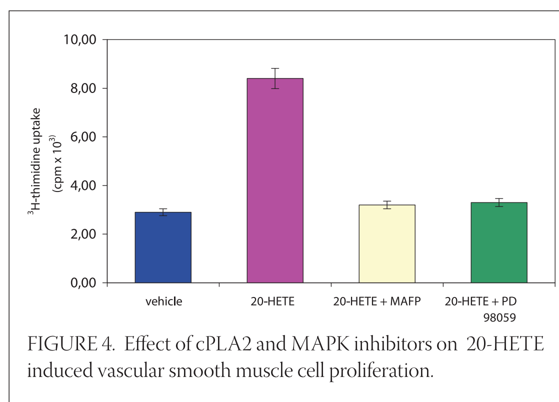

FIGURE 4. Effect of cPLA2 and MAPK inhibitors on 20-HETE induced vascular smooth muscle cell proliferation.

genase, through Ras/MEK/MAPK pathway activation contribute to vascular smooth muscle cell proliferation induced by NE or ANG II (13, 14). ET-1 is vasoactive peptide that acts via ET-A receptors coupling inducing vascular smooth muscle cell contraction. ET-1 is involved in vascular smooth muscle cell growth and in the development and maintenance of hypertension. ET-1 promotes vascular smooth muscle cell proliferation through extracellular signal-regulated kinase activation (2). ET-1 induced arachidonic acid release is mediated by cPLA2 activation in rat tail artery (9). Our study indicates that CYP-450 and LO metabolites of AA, 20-hydroxyeicosatetraenoic acid (HETE) and 12-HETE increased ET-1-induced VSMC proliferation. That the metabolites of arachidonic acid generated via CYP-450 and LO but not COX, induce VSMC proliferation was indicated by finding that exogenous arachidonic acid, like ET-1, increased [^{3}H]thymidine incorpora¬tion into VSMC and this was inhibited by 17-ODYA and baicalein, but not by indomethacin. ET-1 induced VSMC proliferation was independent prostaglandins. The effect of NE and ANG II to stimulate VSMC proliferation has also been reported to be not altered by the COX inhibitors (15). NE, ANG II and arachidonic acid metabolites have been shown to increase MAPK activity (15, 16, 17, 18) in VSMC. Our finding that inhibitors of MAP kinase (PD-98059) and cPLA2 (MAFP) attenuated ET-1 as well as 20-HETE induced VSMC proliferation suggest that ET-1 induced VSMC proliferation is mediated by CYP-450 and LO products of arachidonic acid through cPLA 2 and MAP kinase activation.

Conclusion

The present study demonstrates that ET-1 promotes vascular smooth muscle cell proliferation through activation of cPLA2 resulting in arachidonic acid release. Arachidonic metabolites 12-, 20-HETE generated by LO and CYP-450 induce vascular smooth muscle cell proliferation by activating MAP kinase.

List of Abbreviations

AA	-	arachidonic acid
ANG II	-	angiotensin II
BACL	-	baicalein
COX	-	cyclooxygenase
cPLA2	-	cytosolic phospholipase A2
CYP-450	-	cytochrome P-450
IND	-	indomethacin
HETE	-	hydroxyeicosatertaenoic acid
LO	-	lipoxygenase
MAFP	-	metyl arachidonil fluoro phosphonate
MAP kinase	-	mitogen activated protein kinase
NE	-	norepinephrine
17-ODYA	-	17-octadecynoic acid
VSMC	-	vascular smooth muscle cell

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