Transfection and Functional Expression of CYP4A1 and CYP4A2 Using Bicistronic Vectors in Vascular Cells and Tissues

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ABSTRACT

20-hydroxyeicosatetraenoic acid (20-HETE), a CYP4A-derived arachidonic acid metabolite, is a potent vasoconstrictor and a modulator of vascular reactivity. We have shown that CYP4A1 and CYP4A2 are the major CYP4A isoforms expressed in the rat renal microcirculation. In the present study, we constructed two bicistronic vectors, pIRE2-EGFP-4A1 and pIRE2-EGFP-4A2, and examined their functional efficacy in COS-1 and vascular smooth muscle (A7r5) cells and in microdissected rat interlobar arteries. Immunocytochemistry coupled with fluorescence microscopy of pIRE2-EGFP-4A1- or pIRE2-EGFP-4A2-transfected COS-1 and A7r5 cells indicated that both enhanced green fluorescence (EGFP) and CYP4A1/4A2 were expressed in 80 to 90% of the cells. Western blot analysis showed a 3- to 5-fold increase of CYP4A1 and CYP4A2 proteins in pIRE2-EGFP-4A1- and pIRE2-EGFP-4A2-transfected cells as compared with control pIRE2-transfected cells. Cells transfected with pIRE2-EGFP-4A1 and pIRE2-EGFP-4A2 catalyzed arachidonic acid ω-hydroxylation to 20-HETE at rates of 0.85 ± 0.29 and 0.27 ± 0.04 nmol/10^7 cells/h, respectively. Transfection of interlobar arteries with either plasmid yielded EGFP immunofluorescence that was localized to the intima, media, and adventitia. Arteries transfected with pIRE2-EGFP-4A1 and pIRE2-EGFP-4A2 showed increased vasoreactivity displaying EC_{50} to phenylephrine of 0.24 ± 0.07 and 0.11 ± 0.03 μM, respectively, as compared with arteries transfected with pIRE2-EGFP (1.11 ± 0.21 μM; n = 6, p < 0.05). The increased vasoreactivity to phenylephrine was inhibited by N-methylsulfonyl-12,12-dibromododec-11-enamide, an inhibitor of CYP4A-catalyzed reactions, suggesting that a product of CYP4A1 and CYP4A2 catalytic activity contributed to the increased constritor responsiveness. Removal of the endothelium did not prevent the sensitization to phenylephrine in vessels transfected with the plasmid containing the CYP4A1 cDNA, suggesting that the CYP4A product responsible for the sensitizing effect, presumably 20-HETE, is not of endothelial cell origin.

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ABBREVIATIONS: 20-HETE, 20-hydroxyeicosatetraenoic acid; P450, cytochrome P450; EGFP, enhanced green fluorescence protein; IRES, internal ribosome entry site; DMEM, Dulbecco’s modified Eagle’s medium; PBS, phosphate-buffered saline; HPLC, high-performance liquid chromatography; DDMS, N-methylsulfonyl-12,12-dibromododec-11-enamide; EET, epoxyeicosatrienoic acid.
HETE must take into consideration that it is a lipid molecule. As such, exogenously added 20-HETE may reach targets different from those affected by the endogenously generated 20-HETE since its interaction with the cell membrane is multifaceted; it readily enters the cell, can be esterified to phospholipids, or interact with a surface protein. Consequently, producing 20-HETE within the cell/tissue may shed light on its role as a cellular effector molecule.

A viable approach to increasing 20-HETE in a given cell or tissue is overexpression of P450 proteins that catalyze its formation by gene transfer techniques. CYP4A proteins including CYP4A1, CYP4A2/4A3, and CYP4A8 have been extensively characterized as the arachidonic acid ω-hydroxylases in the rat kidney. We have shown that CYP4A1 and CYP4A2/4A3 are major CYP4A proteins expressed in the rat renal microcirculation and both contribute significantly to vascular synthesis of 20-HETE (Wang et al., 1998b). Accordingly, we constructed two bicistronic vectors, pIRES2-EGFP-4A1 and pIRES2-EGFP-4A2, in which coexpression of EGFP allows assessment of transfection efficiency and detection of transfected CYP4A proteins. In this study, we demonstrate the coexpression of EGFP and CYP4A proteins in COS-1 cells, in A7r5 vascular smooth muscle cells, and in microdissected rat interlobar arteries. We further show that these vectors efficiently drive the expression of CYP4A1 and CYP4A2 by increasing protein levels and 20-HETE synthesis. Furthermore, interlobar arteries transfected with pIRES2-EGFP-4A1 or pIRES2-EGFP-4A2 demonstrated increased constrictor responsiveness to phenylephrine, presumably via increased CYP4A1 and CYP4A2 catalytic activity. The ability to monitor P450 expression by following EGFP immunofluorescence provides a useful tool for evaluating the consequences of increasing endogenous levels of 20-HETE, an important modulator of vascular function.

Materials and Methods

Construction of CYP4A-pIRES2-EGFP Vectors. CYP4A1 cDNA (2130 base pairs), subcloned into the EcoRI and BamHI cloning sites of the pcDNA3.1 vector (Kidae et al., 2003), was released by digestion with EcoRI and BamHI, purified, and then ligated into the pIRES2-EGFP vector (BD Biosciences Clontech, Palo Alto, CA), a 5.3-kilobase vector that contains the internal ribosome entry site (IRES) of the encephalomycocarditis virus between the multiple cloning site and the EGFP coding region. CYP4A2 cDNA (1720 base pairs), originally subcloned into the EcoRI site of PCR II vector, was released by digestion with EcoRI, purified, and then ligated into pIRES2-EGFP vector. Both the CYP4A (cloned into the MCS) and the EGFP are translated separately from a single bicistronic mRNA expressed under the control of the CMV promoter. The orientation of the cloned CYP4A1 and CYP4A2 was verified by restriction analysis, and its identity was confirmed by polymerase chain reaction analysis with isoform-specific primers.

Cell Culture and Transfection. A7r5 and COS-1 cells (American Type Culture Collection, Manassas, VA) were cultured in Dulbecco’s modified Eagle’s medium (DMEM) that was supplemented with 10% fetal bovine serum, 100 U/ml penicillin G, and 10 µg/ml streptomycin. Cultures were maintained in a humidified atmosphere of 5% CO2 in air at 37°C. The medium was changed every 2 days, and cells were passaged with Trypsin-EDTA at least once a week.

Cells were washed in PBS, fixed with 90% ethanol for 10 min at room temperature, and rinsed with PBS three times. The fixed cells were then permeabilized with a blocking solution consisting of 2% bovine serum albumin and 0.05% Tween 20 in PBS for 1 h. After washing with PBS three times, the cells were incubated with goat anti-rat CYP4A1 antibody (1:100; BD Gentest, Woburn, MA), which cross-reacts with all CYP4A proteins, and with mouse anti-EGFP monoclonal antibody (1:100; JL-8, Invitrogen) in blocking solution for 1 h at room temperature. After further washing with PBS, slides were incubated for 1 h at room temperature with a secondary antibody labeled with Alexa Fluor 488-conjugated goat anti-mouse IgG (Molecular Probes, Eugene, OR) for CYP4A1 (1:1000) and a secondary antibody labeled with Alexa Fluor 594-conjugated rabbit anti-goat IgG (Molecular Probes) for EGFP (1:300). Control experiments included omission of primary antibodies and addition of nonimmune rabbit and mouse serum for CYP4A and EGFP, respectively.

Microdissection and Transfection of Renal Interlobar Arteries. Sprague-Dawley rats were anesthetized with pentobarbital sodium (50 mg/kg b.wt.). Kidneys were excised, and interlobar arteries were microdissected and freed from cortical and connective tissue under a microscope as previously described (Kidae et al., 2003). Isolated rat interlobar arteries (10 vessels/dish) were incubated with a mixture containing 30 µg of CYP4A2-pIRES2-EGFP or CYP4A1-pIRES2-EGFP vector and 30 µl of Lipofectamine Reagent for 6 h in 200 µl of Opti-MEMI medium in a humidified atmosphere of 5% CO2 in air at 37°C, after which 1 ml of DMEM with 10% FBS was added and incubation proceeded for an additional 18 h. Arteries were fixed in 4% formaldehyde in phosphate-buffered saline, dehydrated in ethanol, permeated in xylene, embedded in OCT (Sakura Finetek Europe, Zoeterwoude, the Netherlands), and sectioned 5 µm thick. Deparaffinized and rehydrated sections were stained with anti-EGFP antibodies (1:100) and anti-CYP4A antibodies (1:100) followed by secondary antibodies as described above. Control experiments included omission of primary antibodies and addition of nonimmune rabbit and mouse serum for CYP4A and EGFP, respectively. Immunostaining was visualized by confocal microscopy.

Western Blot Analysis. A7r5 or COS-1 cells (T-175; 50% confluent) were transfected with a mixture containing 25 µg of CYP4A1 or CYP4A2 recombinant plasmids and 25 µl of Lipofectamine as described above. Cells were harvested using cell lysis buffer (0.1 M potassium phosphate buffer, 0.25 M sucrose, 1 mM EDTA, 0.1% NP-40, 0.1 mM phenylmethylsulfonyl fluoride, and Sigma protease inhibitor cocktail 1:1000, pH 7.4). The lysates were centrifuged at 4000 g for 10 min, and the protein concentration in supernatant was measured using the BioRad assay. Microdissected interlobar arteries were snap-frozen and homogenized in 10 mM potassium phosphate buffer, pH 7.2, containing 25 mM sucrose, 0.1% NP-40, 1 mM EDTA, and 0.1 mM phenylmethylsulfonyl fluoride. The homogenized arteries were centrifuged at 4000 g for 15 min, and the supernatant was used for Western blot analysis. Proteins (10–20 µg) were separated by SDS/polyacrylamide gel electrophoresis at 25 mA and 4°C for 18 to 20 h and were transferred electrophoretically to a nitrocellulose membrane. Immunoblotting was performed as described previously (Marji et al., 2002) using the following primary and secondary antibodies: goat anti-rat CYP4A1 polyclonal antibody (1:1000; Gentest), mouse anti-EGFP monoclonal antibody (1:1000; Invitrogen), rabbit...
anti-goat IgG (1:2000), and goat anti-mouse IgG (1:1000). Immuno-
reactive proteins were detected using the ECL Plus detection system
(Amersham Biosciences Inc. (Piscataway, NJ) according to the manu-
facturer’s instructions. The anti-CYP4A1 polyclonal antibody cross-
reacts with all CYP4A isofoms, i.e., CYP4A2, CYP4A3, and
CYP4A8 (Nguyen et al., 1999).

**Measurement of Enzyme Activity.** Control and transfected
cells were washed three times with DMEM and incubated with [1-14C]-
lauric acid or [1-14C]-arachidonic acid (2 µCi) in 5 ml of Opti-
media for 2 to 6 h at 37°C. Aliquots from the culture medium (2 ml)
were taken at 2, 4, and 6 h after addition of the radiolabeled sub-
strates. In some experiments, microsomes were prepared by centrifu-
gation. Briefly, cells were scraped and homogenized in 1 ml of 0.25
M sucrose containing 1 mM EDTA. The homogenate was centrifuged
at 1000g for 5 min and the supernatant collected and further centri-
fuged at 100,000g for 60 min. The 100,000g pellet was resuspended
in 50 mM potassium phosphate buffer, pH 7.5, containing 0.2 mM
EDTA and 20% glycerol and stored at -80°C until used. The
100,000g pellet (100–300 µg of protein) were incubated with
[1-14C]-arachidonic acid or [1-14C]-lauric acid (0.4 µCi, 7 nmol) and
NADPH (1 mM) in 0.3 ml of potassium phosphate buffer (100 mM,
pH 7.4) containing 10 mM MgCl2 for 30 min at 37°C. The reaction
was terminated by acidification, and the metabolites were extracted
with ethyl acetate and separated by HPLC as previously described
(Marji et al., 2002).

**Measurements of 20-HETE Levels.** Interlobar arteries were
transfected with the various plasmids as described above and incu-
bated for an additional 24 h. The medium was collected, and [3H]-
20-HETE (0.5 ng/ml) was added as an internal standard. 20-
HETE was isolated by ethyl acetate extraction and HPLC separation
and was further derivatized to the pentafluorobenzyl ester, trimethyl-
silyl ether. Negative chemical ionization-gas chromatography/mass
spectrometry was performed on an HP6890 mass spectrometer
(Hewlett Packard, Palo Alto, CA) interfaced with a capillary gas
chromatographic column (HP-5MS, 30 m × 0.25 mm × 0.25 µm;
Agilent Technologies, Palo Alto, CA). Single ions were monitored: m/z
391 and 393 for the derivatized 20-HETE and [2H2]20-HETE,
respectively. 20-HETE levels were quantified as described previ-
ously (Marji et al., 2002).

**Measurement of Isometric Tension in Vascular Rings.**
Interlobar arteries (~230 µm, internal diameter) were cut into ring
segments (2 mm). Rings were mounted on wires in the chambers of
a multivessel myograph (JP Trading, Aarhus, Denmark) filled with
Krebs’ buffer (37°C) gassed with 95% O2/5% CO2. After 30 to 60 min
of equilibration, the vessels were set to an internal circumference
equivalent to 90% of that which they would have in vitro when
terlobar arteries (Buus et al., 2000). Endothelium removal was further confirmed
by the lack of endothelium-dependent relaxation to acetylcholine (1
µM) in phenylephrine (10−5 M)-preconstricted arteries.

**Data Analysis.** Data are expressed as mean ± S.E.M. Concen-
tration-response data derived from each vessel were fitted separately
to a logistic function by nonlinear regression. The maximum asymm-
tote of the curve and the concentration of agonist producing 50% of
the maximal response (EC50) were calculated using commercially
available software (Prism 2.01; GraphPad Software Inc., San Diego,
CA). Concentration-response data were analyzed by a two-way anal-
yses of variance followed by a Duncan multiple range test. Other data
were analyzed by a Student’s t test for paired or unpaired observa-
tions as appropriate. The null hypothesis was rejected at p < 0.05.

**Results**

CYP4A protein expression was undetectable in both the kidney-derived COS-1 cells and the vascular smooth muscle-
derived cell line, A7r5. Immunocytochemistry of A7r5 cells
transfected with pIRE2-EGFP-4A1 showed a coexpression of
EGFP and CYP4A1 immunoreactive proteins (Fig. 1). Likewise, EGFP and CYP4A2 immunoreactive proteins were
coexpressed in A7r5 cells transfected with pIRE2-EGFP-4A2 (Fig. 1). Similar results were obtained in COS-1 cells
(data not shown). The transfection efficiency of these vectors
was estimated to be about 90% in both cell types. Western
blot analysis using antibodies against CYP4A and EGFP
showed the presence of immunoreactive CYP4A and EGFP
proteins only in cells transfected with pIRE2-EGFP-4A1 or
pIRE2-EGFP-4A2 but not in cells transfected with the empty
vector, pIRE2 (Fig. 2). The increased levels of CYP4A1
and CYP4A2 proteins in cells transfected with pIRE2-EGFP-4A1
and pIRE2-EGFP-4A2, respectively, were associated with
increased catalytic activity characteristics of these proteins.
Figure 3 depicts representative HPLC patterns of metabo-
lites obtained following incubation of transfected COS-1 cells
with arachidonic acid. Cells expressing the CYP4A1 protein

![Fig. 1. Coexpression of CYP4A1, CYP4A2, and EGFP in A7r5 cells transfected with pIRE2-EGFP-4A1 (left panel) and pIRE2-EGFP-4A2 (right panel). Representative confocal micrographs in which green fluo-
rescence of EGFP was detected with the fluorescein isothiocyanate filter and CYP4A fluorescence with the Texas red filter: a and d, green fluo-
rescence protein; b and e, CYP4A; c and f, EGFP and CYP4A.](image-url)
metabolized arachidonic acid to 20-HETE (Fig. 3A), whereas cells expressing the CYP4A2 protein produced 19-HETE, 20-HETE, and 11,12-EET (Fig. 3B) similar to what has been shown for the Sf9-derived recombinant protein (Wang et al., 1996; Nguyen et al., 1999). The rate of 20-HETE production in cells expressing CYP4A1 was $0.85 \pm 0.29 \text{ nmol/10}^7 \text{ cells/h}$. Cells transfected with CYP4A2 metabolized arachidonic acid to 19/20-HETE and EET at a rate of $0.27 \pm 0.04$ and $0.07 \pm 0.03 \text{ nmol/10}^7 \text{ cells/h}$, respectively. Cells treated with the control vector (pIRES2-EGFP) did not metabolize arachidonic acid (Fig. 3C).

Similar results were obtained when lauric acid, a known substrate of CYP4A proteins, was used as the substrate. As seen in Fig. 4, CYP4A1-transfected cells metabolized lauric acid primarily to the 12-OH-lauric acid at a rate of $1.66 \pm 0.41 \text{ nmol/10}^7 \text{ cells/h}$ (Fig. 4A) as did CYP4A2-transfected cells, which also exhibited significant $\omega$-1 hydroxylation of lauric acid (Fig. 4B). CYP4A2-transfected cells metabolized lauric acid to 12-OH-lauric acid at a rate of $0.76 \pm 0.32 \text{ nmol/10}^7 \text{ cells/h}$. Cells that were treated with the control vector (pIRES2-EGFP) did not metabolize lauric acid (Fig. 3C).

Using confocal immunofluorescence histocytometry techniques, we examined the colocalization of EGFP and CYP4A in interlobar arteries transfected in vitro with pIRES2-EGFP-4A1 and pIRES2-EGFP-4A2. As seen in Fig. 5, EGFP and CYP4A immunofluorescence in pIRES2-EGFP-4A1- and pIRES2-EGFP-4A2-transfected arteries was apparent in all vascular layers including the intima, media, and adventitia. Merging the two images shows that these two proteins are colocalized within the transfected arteries (Figs. 5, C and F). Western blot analysis was further performed to document an increase in the levels of CYP4A immunoreactive proteins following transfection of interlobar arteries with these plasmids. Transfection with pIRES2-EGFP-4A1 and pIRES2-EGFP-4A2 increased CYP4A immunoreactive proteins to levels higher than the control cultured arteries or arteries cultured with the control plasmid, pIRES2-EGFP (Fig. 6, A and B). In addition, the amount of 20-HETE in the culture medium of vessels transfected with pIRES2-EGFP-4A1 and pIRES2-EGFP-4A2 was 1.5- and 1.7-fold higher, respectively, than the amount in the medium of vessels transfected with the control plasmid, pIRES2-EGFP (Fig. 6C). To further evaluate whether the bicistronic vectors described in this study are useful for functional studies, we examined vascular reactivity to phenylephrine in interlobar arteries transfected with pIRES2-EGFP-4A1, pIRES2-EGFP-4A2, and pIRES2-EGFP. As seen in Fig. 7A, the concentration-response curves to phenylephrine in arteries transfected with pIRES2-EGFP-4A1 and pIRES2-EGFP-4A2 were shifted to the left, resulting in EC$_{50}$ values that are, respectively, 20 and 10% of the EC$_{50}$ in arteries transfected with the control vector, pIRES2-EGFP. The maximal response to phenylephrine was not different between arteries transfected with pIRES2-EGFP-4A1 and pIRES2-EGFP-4A2, or pIRES2-EGFP (Fig. 7A). Pretreatment with DDMS, a selective inhibitor of CYP4A-catalyzed arachidonic acid $\omega$-hydroxylation (Wang et al., 1998a), increased the EC$_{50}$ to phenylephrine in arteries transfected with the control

Fig. 2. Western blot analysis of CYP4A immunoreactive proteins in A7r5 and COS-1 cells transfected with pIRES2-EGFP-4A1 (A) and pIRES2-EGFP-4A2 (B). CYP4A and EGFP immunoreactive proteins in microsomal fractions from cells transfected with the plasmids pIRES2-EGFP-4A1, pIRES2-EGFP-4A2, or pIRES2-EGFP were detected by Western blot analysis as described under Materials and Methods. Lane 1, corresponding EGFP, CYP4A1, and CYP4A2 protein standards; lane 2, cells transfected with the empty vector pIRES2; lane 3, cells transfected with pIRES2-EGFP-4A1 or pIRES2-EGFP-4A2.

Fig. 3. Arachidonic acid metabolism in pIRES2-EGFP-4A1- and pIRES2-EGFP-4A2-transfected cells. Representative HPLC profiles are depicted for cells transfected with pIRES2-EGFP-4A1 (A), pIRES2-EGFP-4A2 (B), and pIRES2-EGFP (C).
pIRES2-EGFP from 1.11 ± 0.21 to 3.25 ± 0.75 μM, further substantiating previous reports that a product of CYP4A enzymes contribute to constrictor responsiveness in these arteries (Kaide et al., 2003). Likewise, pretreatment with DDMS caused a rightward shift in the concentration-response curves to phenylephrine in pIRES2-EGFP-4A1- and pIRES2-EGFP-4A2-transfected arteries, increasing the EC50 by about 20-fold to values not different from the EC50 of vessels transfected with the control vector pIRES2-EGFP and pretreated with DDMS. DDMS did not affect the maximal response to phenylephrine in any of these vessels (Fig. 7B).

Since immunostaining showed the presence of immunoreactive protein in the endothelial layer, we examined whether removal of the endothelium altered vascular response to phenylephrine. The results depicted in Fig. 8 indicate that removal of the endothelium sensitized vessels transfected with either pIRES2-EGFP or pIRES2-EGFP-4A1 to phenylephrine, resulting in a 2-fold reduction of the EC50 values relative to corresponding values in vessels with endothelium. Removal of the endothelium did not interfere with the sensitizing effect of CYP4A1 transfection. The contractile response to 80 mM KCl was not affected by removal of the endothelium (viz., 1.88 ± 0.19 versus 1.50 ± 0.26 mN/mm in intact and denuded pIRES2-EGFP-transfected arteries and 1.18 ± 0.10 versus 0.94 ± 0.10 mN/mm in intact and denuded pIRES2-EGFP-4A1-transfected arteries).

Discussion

A common feature of most primary cultures or established cell lines is the lack of measurable P450 levels and catalytic activity (Sirica and Pitot, 1979; Paine, 1991; Lin et al., 1995). Thus, transfection of P450 genes provides a useful tool for functional analysis of metabolic pathways. Chen et al. (1999) demonstrated successful stable transfection of the renal epithelial cell line, LLCPKe14, with an active 14(S),15(R)-arachidonate epoxygenase of bacterial origin containing a P450 domain fused to a CPR domain. These authors further demonstrated that the 14(S),15(R)-ETE functions as an intracellular second messenger in response to EGF. Endothelial cells transfected with CYP2C epoxygenases were instrumental in elucidating the role of EETs because EDHF and cellular mechanisms underlie their effect on cell proliferation and angiogenesis (Fisslthaler et al., 1999; Fleming et al., 2001; Michaelis et al., 2003). The use of bicistronic vectors in this study provides the means to estimate transfection efficiency.
and to trace and localize the expression of the transfected CYP4A within the tissue. The results show that both GFP and CYP4A proteins are coexpressed in cultured cells and in freshly isolated and cultured renal interlobar arteries. The increased expression of CYP4A1 or CYP4A2 using these bicistronic vectors was associated with increased \( \text{H}_{\text{9275}} \)-hydroxylation of lauric acid, the preferred substrates of CYP4A enzymes, and arachidonic acid, as well as with increased constrictor responsiveness to phenylephrine.

In COS-1 and A7r5 cells, transfection efficiency was about 95% as estimated by GFP fluorescence. These cells do not contain measurable CYP4A1 or CYP4A2 proteins, nor do they metabolize arachidonic acid to 20-HETE. Transfection of the bicistronic plasmids yielded expression of both proteins as measured by confocal immunofluorescence cytochemistry and by Western blotting. The transfected CYP4A1 and CYP4A2 exhibited their characteristic activity as \( \omega \)-hydroxylases of lauric and arachidonic acids. Cells transfected with the CYP4A1-containing plasmid metabolized arachidonic acid to 20-HETE at the higher rate than cells transfected with the CYP4A2-containing plasmid, consistent with the finding that CYP4A1 is the most efficient arachidonic acid \( \omega \)-hydroxylase among the CYP4A isoforms (Okita and Okita, 2001).

Interlobar arteries are an important site of 20-HETE synthesis and function. These arteries (~200-μm diameter) produce a significant amount of 20-HETE (Marji et al., 2002). Moreover, constrictor responsiveness to phenylephrine is attenuated in the presence of CYP4A inhibitors, such as DDMS, and is greatly magnified in the presence of exogenously added 20-HETE (Kaide et al., 2003), thus implicating 20-HETE as an endogenous modulator of constrictor responses. The results of this study clearly showed that the levels of immunoreactive CYP4A protein in these arteries can be increased as a result of transfection with expression plasmids containing the CYP4A1 or CYP4A2 cDNA. In examining the consequences of increased expression on vascular reactivity in renal interlobar arteries, we showed that both CYP4A1- and CYP4A2-transfected arteries exhibit enhanced responsiveness to phenylephrine, consistent with the findings in other vascular beds (Marji et al., 2002).
increased vascular reactivity to phenylephrine in vessels overexpressing CYP4A is, in part, the result of increased \( \omega-1 \) hydroxylation of EETs with attendant reduction of the EETs bioactivity. Although vessels transfected with CYP4A1 and CYP4A2 expressed and produced comparable levels of immunoreactive CYP4A proteins and 20-HETE, respectively, CYP4A2-transfected vessels showed greater sensitivity to phenylephrine than CYP4A1-transfected vessels. This difference may be explained by differences in their catalytic activity and/or their ability to degrade EETs as described above.

The present study demonstrates significant levels of CYP4A immunoreactivity in the endothelial layer of the renal interlobular arteries transfected with \( \pi \)RES2-EGFP-4A1 or \( \pi \)RES2-EGFP-4A2. However, removal of the endothelium did not prevent the sensitization to phenylephrine in vessels transfected with the plasmid containing the CYP4A1 cDNA. This observation implies that the CYP4A product responsible for sensitization to phenylephrine in vessels transfected with CYP4A-containing plasmids is not of endothelial cell origin. Endothelial removal increased the sensitivity to phenylephrine in vessels transfected with either \( \pi \)RES2-EGFP-4A1 or the control plasmid, \( \pi \)RES2-EGFP. It is likely that endothelial denudation excludes factors such as NO, prostaglandin \( \mathrm{I}_2 \), EETs, and lipoygenase-derived eicosanoids (Li et al., 1997; Campbell and Harder, 1999; Campbell et al., 2003) that counteract vasoconstrictor mechanisms.

References


Buus NH, Simonsen U, Pilegaard HK, and Mulvany MJ (2000) Nitric oxide, prostaglandin and the sensitivity to phenylephrine. An additional possibility is sug- gested by observations that EETs are excellent substrates for CYP4A metabolism. Hence, since CYP4A-catalyzed reactions, to offset this increase suggests that increased vascular reactivity to phenylephrine in vessels transfected with the control plasmid, \( \pi \)RES2-EGFP, \( \dagger \), \( p < 0.05 \) from endo- thelial denuded vessels transfected with \( \pi \)RES2-EGFP; \( \ddagger \), \( p < 0.05 \) from corresponding vessels with the endothelium.

\[ \text{Phenylephrine (log M)} \]

Fig. 8. Concentration-dependent constrictor responses to phenylephrine in the absence and presence of endothelium in CYP4A1-transfected renal interlobar arteries. Results are expressed as the percentage of KCl response and are the mean ± S.E., \( * \), \( p < 0.05 \) from intact vessels transfected with the control plasmid, \( \pi \)RES2-EGFP; \( \dagger \), \( p < 0.05 \) from endo- thelial denuded vessels transfected with \( \pi \)RES2-EGFP; \( \ddagger \), \( p < 0.05 \) from corresponding vessels with the endothelium.

\[ n \text{ EC}_{50}(\mu M) \]

\[ \begin{array}{c|c|c}
\hline
6 & 1.57 ± 0.27 & * \\
6 & 0.69 ± 0.25 & \dagger \\
6 & 0.09 ± 0.02 & * \\
7 & 0.04 ± 0.01 & * \\
\hline
\end{array} \]

\[ \text{KCl} \]

\[ \% \text{ of KCl Response} \]

\[ \begin{array}{c|c|c}
\hline
9 & 250 & \\
18 & 200 & \\
27 & 150 & * \\
36 & 100 & * \\
45 & 50 & * \\
\hline
\end{array} \]
cross-talk with the epidermal growth factor receptor (EGFR). FASEB J 17:770–772.


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