Cytochrome P450 Induced Differentially in Endothelial Cells Cultured from Different Organs of *Anguilla rostrata*

Rita Anne Garrick, Bruce R. Woodin and John J. Stegeman

Department of Natural Sciences, Fordham University, 113 w. 60th Street, New York, NY 10023, USA (R.A.G.) and Department of Biology, Woods Hole Oceanographic Institution, Woods Hole MA 02543, USA (B.R.W., J.J.S. and R.A.G.)

Contact author: Rita Anne Garrick

                     Dept. of Natural Sciences

                     Fordham University

                     113 W. 60th Street

                     New York, NY 10023 USA

                     212-636-6333

                     212-636-7213 FAX

                     rgarrick@whoi.edu

Running title: Eel endothelial cells

Abstract:

Endothelial cells are a structural barrier and an active regulator of many bodily processes. CYP1A activity is induced in the endothelium of teleosts and mammals exposed to lipophilic xenobiotics, such as polycyclic aromatic hydrocarbons, and can have significant consequences for endothelial functions. We exposed cultures of characterized endothelial cells from the heart, kidney and rete mirabile of the eel, *Anguilla rostrata*, to AhR agonists. In heart endothelial cells the maximum response (based on EROD activity) to TCDD, 113 pmol/mg-min, was at 1 nM TCDD and the peak response to βNF, 135 pmol/mg-min, was at 3 μM βNF. The maximum response to TCDD in the kidney endothelial cells is 12 pmol/mg-min at 0.3 nM TCDD. The rete mirabile capillary endothelial cells responded minimally or not at all to exposure to TCDD and βNF. Both the heart and kidney endothelial cells (but not the rete mirabile capillary cells) have a low level of EROD activity (12.7 and 5.2 pmol/mg-min respectively) in untreated or DMSO-treated cells. The robust response of the heart endothelial cells to induction and the lack of response in the rete mirabile capillary endothelial cells indicate that these cells are a good resource to use to investigate the physiological consequences of AhR agonist exposure and CYP1A induction in different areas of the vasculature.

Key words: teleost, eel, fish, microvasculature, endothelium, pHAH, cytochrome P450, EROD, dioxin

INTRODUCTION

Endothelial cells that line the vascular systems in vertebrates function as a structural barrier between the blood and tissues and as an active regulator of many bodily processes. Compromising the structural barrier affects the exchange of materials, e.g., gases, solutes or ions, between the blood and tissues. Regulatory functions of endothelial cells also can be altered by a large number of blood-borne chemicals as well as mechanical stimuli. Although endothelium is structurally similar throughout the vasculature not all endothelia respond to the same stimuli. *In vivo* studies show that endothelium in different types of vessels or in different organs can differ in specific regulatory processes as secretion of vasoactive agents (Steinhorn et al.,1993; Gannon et al.,2000) and cultures of endothelial cells have been used to elucidate many of these regulatory processes (Lou et al.,1997; Sadar and Andersson,2001)The endothelium has also been implicated as the site of origin of pathological processes, i.e., atherosclerosis, and the target for diverse toxic or mutagenic agents (Annas et al.,2000; Dugas et al.,2000). Here we address functional distinctions in endothelial cell response to toxicants.

Exposure to lipophilic xenobiotics, such as polycyclic aromatic hydrocarbons (PAH) or planar halogenated aromatic hydrocarbons (pHAH) can produce chronic or acute responses that are either harmful or protective in vertebrates (Nebert et al.,2000; Thum et al.,2000; Mimura and Fujii-Kuriyama,2003). For example, exposure to PAH and pHAH produces a number of responses that include edema (Teraoka et al.,2003), developmental abnormalities (Guiney et al.,1997; Cantrell et al.,1998), mutagenesis (Shimada and Fujii-Kuriyama,2004) and altered responses to endogenous signaling

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molecules (Annas and Brittebo,1998; Schlezinger et al.,1998). The effects seen in the cardiovascular system (e.g., atherosclerosis, edema) may indicate damage to the endothelial layer (Bayou-Denizot et al.,2000; Hennig et al.,2002b).

Exposure to PAH or pHAH elicit the induction of enzymes of the cytochrome P4501A (CYP1A) family through the aryl hydrocarbon receptor (AhR) transcription factor (Hahn,1998; Mimura and Fujii-Kuriyama,2003). The endothelium in many tissues is rich in AhR expression and responds to xenobiotic exposure with the induction of CYP enzymes (Stegeman et al.,1987; Stegeman et al.,1989; Smolowitz et al.,1991; Stegeman et al.,1991). Thus, endothelial cells in tissue sections from mammals and fish exposed in the environment or in the laboratory show induction of CYP1A enzymes (Smolowitz et al.,1991; Stegeman et al.,1991; Schlezinger and Stegeman,2000; Schlezinger et al.,2000b) . Similarly, cultures of mammalian endothelial cells show induction of CYP1A by pHAH and are being used to study the mechanisms of the cellular response of the endothelium to xenobiotics (Stegeman et al.,1995; Celander et al.,1997; Hennig et al.,2002).

The ubiquitous distribution of many PAH and pHAH compounds in marine and freshwater environments has led to intensive study of how aquatic organisms, particularly fish, respond to these compounds (Hahn et al.,1998; Schlezinger et al.,2000b; Schlezinger and Stegeman,2000b). Induction of fish CYP1A by AhR agonists have been studied in highly exposed natural populations, in laboratory studies of fish taken from the field, in fish embryos and in fish hepatoma cells (Hahn et al.,1993; Guiney et al.,1997; Schlezinger and Stegeman,2000b). With the establishment of methods to maintain and subculture teleost fish endothelial cells (Garrick,2000) we can

now address questions about effects of xenobiotics on the endothelium that could not be tested previously.

We report here that endothelial cells isolated from different organs of the eel, *Anguilla rostrata*, respond to exposure to PAHs with induction of CYP1A in tissue-specific ways that reflect the pattern seen in these tissues *in vivo*.

MATERIALS AND METHODS

Chemicals

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) (>98% pure) was obtained from Ultra Scientific, Hope, RI, USA. 7-Ethoxyresorufin was purchased from Molecular Probes (Eugene, OR). Dimethylsulfoxide (DMSO), β-napthoflavone (βNF) and all other reagents were the highest grade available from Sigma (St. Louis, MO). Cell culture materials from Sigma were cell culture tested grade. Other sources were noted below.

Isolation and Culture of Endothelial Cells

Healthy, adult female eels were obtained from the Marine Resources Center at the Marine Biological Laboratory (Woods Hole, MA). Isolation and cell culture was as described in Garrick,2000. Briefly, the organs were excised, rinsed free of blood and treated to separate the cells. In the rete mirabile the "heart" and "bladder" poles were removed which leaves only the capillaries for isolation. The capillaries were digested enzymatically and plated into T-25 flasks. The excised heart and kidney were cleaned, any large vessels removed and the tissue minced and dispersed into T-25 flasks. All were grown in M199SE [Medium 199 with Earle's salts and 20 mM NaCl, 20 mM HEPES, 26 mM $NaHCO_3$, 0.68 mM glutamine, 10 μM pyruvate 1X antibiotic-antimycotic

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solution (100 U/ml penicillin, 100 μM/ml streptomycin, 2.5 μM/ml amphotericin B), and

50 μg/ml gentamicin] with 20 % serum (Cosmic Calf Serum, fortified (CCS), Hyclone,

Logan, Utah), 20 μg/ml of endothelial cell growth supplement (ECGS) and 50 μg/ml

heparin (Sigma, cell culture tested) in T-25 Primaria ® flasks coated with 10 μg of

fibronectin and 1 μg/ml of hyaluronic acid (FHA) and incubated at 25 ° C in humidified

air with tightly capped non-vented tops. At confluence the cells were subcultured or

aliquots frozen by standard procedures. All cells used in this study were in passage 1 to

3.

The endothelial origin of the cells was confirmed by the uptake of fluorescently

labeled acetylated low density lipoprotein (DiI-Ac-LDL, 1,1'-dioctadecyl-1-2,3,3',3'-

tetramethyl-indocarbocyanide perchlorate acetylated LDL, Biomedical Technologies,

Stoughton, MA) (Garrick, 2000). Cells were grown under standard conditions in coated

slide flasks, incubated with DiI-Ac-LDL and viewed with a Zeiss Axiovert S100

fluorescent microscope.

<u>Treatment of Cells</u>

Cells were plated into coated (FHA) 48 well plates (Costar™, Cambridge MA) at a

density of ~5 -10 x 10^4 cells/well and grown to confluence in 0.5 ml of M199SE with 20

% CCS and ECGS. The plates were sealed with 2 layers of airtight plastic plate sealer

(Secureseal Therm Seal®, Fisher Scientific, Springfield NJ), covered and incubated at

25° C in humidified air. At confluence the medium was removed and the cells were

rinsed with serum – free M199SE and then 0.5 ml of M199SE with 1% CCS was added

to each well. Cell cultures were treated with TCDD or βNF, dissolved in DMSO (Hahn et

al.,1996), or DMSO alone or with buffer as described previously (Stegeman et al.,1995).

Aliquots (2.5 μl) were added to the wells from dilutions of stock dosing solutions so that DMSO was present at 0.5% (v/v) in all wells except the untreated cells. Each dose was added to 6 wells, the plate was shaken for 1 min. and the cultures returned to the incubator at 25° C in humidified air for 48 hours. The wells were examined microscopically before and after induction to confirm the cells were confluent and intact. Induction of cells for microsome preparations was performed in FHA coated T-75 flasks with a 5 ml of M199SE with 1% CCS with the addition of 25 μl of dosing solution.

Assays

1. EROD activity

The O-deethylation of 7-ethoxyresorufin to resorufin, (EROD) a measure of CYP1A activity, was determined fluorometrically with a Cytofluor 2300 (Millipore) multiwell plate reader by procedures described and then modified by (Kennedy et al.,1995; Hahn et al.,1996; Ganassin et al.,2000). Modifications of incubation procedures follow previously published protocols (Hestermann et al.,2000; Hestermann et al.,2002). At the end of the dosing time the medium was removed and the cells washed with 0.5 ml of phosphate - buffered – saline (PBS) with HEPES pH 7.5 (0.136 M NaCl, 8.1 mM Na_2HPO_4, 1.47 mM K_2HPO_4, 2.68 mM KCl, 10 mM HEPES).

The EROD reaction was initiated by the addition of 200 μl / well of a solution of 7-ethoxyresorufin (2.67 μM in PBS-HEPES at pH 7.8) and monitored kinetically for 20 min. Resorufin standard curves were prepared in PBS-HEPES pH 7.8 in wells in each plate. At completion of the EROD assay total protein content was measured in each well with the fluorescamine assay (Lorenzen and Kennedy,1993) with BSA solutions for the

standard curve. EROD activity was calculated as pmol/mg protein/min of resorufin produced as described in (Hahn et al.,1996; Ganassin et al.,2000)

2. Preparation of microsomes from endothelial cells

Microsomes were prepared from endothelial cells induced in T-75 flasks. After a 48 hour exposure, the dosing medium was removed and the flask was rinsed with serum free M199SE. One milliliter of PBS-HEPES pH 7.5 with 2 mM EDTA was added to the flask. The flask was floated on liquid nitrogen and stored at $-80°$ C until used. Frozen cells were scraped from the flask into homogenization buffer, homogenized, sonicated and separated by differential centrifugation as described in Stegeman et al.(1995). Pellets were resuspended in buffer (50 mM Tris, pH 7.4, 1 mM dithiothreitol, 1 mM EDTA, 20% glycerol) and frozen in liquid N_2 until used. Protein content was determined using the bicinchoninic acid method, with serum albumin as a standard (BCA $^®$ Protein Assay Kit, Pierce, Rockland,IL).

3. Immunoblotting for CYP 1A

The presence of CYP1A was detected by immunoblot analysis of microsomal preparations as described previously with slight modification (Hahn et al.,1993; Stegeman et al.,1995). Microsomal protein (30 to 70 μg) and a range of standards of CYP1A from scup (*Stenotomus chrysops*) were resolved on a 4-20 % acrylamide gradient gel in TRIS-glycine with SDS (Jule,Inc, Milford, CT). The proteins were electrophoretically transferred to a 0.2 μm nitrocellulose membrane (Schleicher & Schuell, Keene, NH) and incubated with antibody Mab 1-12-3 against scup CYP1A (Park et al.,1986; Morrison et al.,1995) with secondary antibody linked to horseradish peroxidase. Mab 1-12-3 strongly recognizes CYP1A1 but not CYP1A2 in mammals.

Detection was with the Amersham Enhanced Chemiluminescence Detection Kit using

Kodak X-AR film. The band intensities of the standards and samples were quantified by

video image analysis (NIH Image).

4. $\underline{EC_{50}}$ Calculation

EC_{50}, the inducer concentration producing half maximal induction of EROD

activity, was calculated using the curve fitting routine of Graph Pad Prism for nonlinear

regression sigmoidal dose response. The modified Gaussian equation for EC_{50} was

described by (Kennedy et al.,1993).

RESULTS:

Cell preparations.

Figure 1 shows cultures of endothelial cells from microvasculature of the kidney

(A,B), capillaries of the rete mirabile (C,D) and the endocardium of the eel heart (E,F).

Each of the cell preparations has a slightly different morphology (Fig 1 B, D, F). At one

time, the cobblestone appearance seen in large vessel cells was considered

characteristic of all endothelial cells but it is now recognized that appearance can vary

for cells from different vessels or with different growth surfaces (Garrick,2000). The

uptake of DiI-Ac-LDL as seen in Fig 1 A, C, and E, confirms that these are endothelial

cells (Garrick,2000). All of the flasks or plates used for the endothelial cells were

coated with FHA. The heart endocardial cells will grow without FHA but at a much

slower rate and microvascular and capillary cells require fibronectin for growth (Garrick,

2000). We found no difference in the total protein measurement if FHA was present or

not. The cells in the 48 well plates were intact and viable after treatment with DMSO or with inducers added in DMSO.

<u>Figure 1 here</u>

<u>Induction of CYP1A activity</u>

The heart, kidney and rete mirabile endothelial cells were plated into 48 well plates and treated for 48 hours with DMSO, TCDD or βNF as described in the Methods. The induction medium contained 1% serum to minimize loss of inducer to serum proteins (Hestermann et al.,2000). At the end of 48 hrs, the EROD activity (CYP1A activity) and total protein were measured in each well as described in the Methods and given in Table 1. The results with each plate are reported in a separate row in Table 1. In each plate a given concentration of inducer is present in six wells; therefore there is a sample size of six, except when noted otherwise.

<u>Table 1 here</u>

The results in Table 1 demonstrate that the three types of endothelial cells respond differently to CYP1A inducers. Both TCDD and βNF induced EROD activity in the heart endothelial cells. Induction of CYP1A activity by TCDD was much higher in the heart endocardial cells than in kidney microvascular endothelium. The capillary cells from the rete mirabile responded minimally or not at all to TCDD or βNF. The basal levels of EROD activity (DMSO-treated cells) were the same in separate experiments for each cell type, i.e., 12.7, 5.2 and 0.05 pmol/mg-min for heart, kidney and rete mirabile, respectively. There was variation in the basal level and amount of enzyme activity induced by a specific TCDD concentration in different plates of cells for a specific cell type (Table 1). Variation in results from individual cultures is often seen in

studies of induction of CYP1A or related enzymatic activities in cells in culture, whether in endothelial cells, hepatic cells or other types of cells (Farin et al.,1994; Sadar and Andersson,2001; Tom et al.,2001).

Figure 2 here

EROD rates for all of the samples at a given inducer concentration in different plates of each type of cell were averaged and plotted in Figures 2A and 2B. Figure 2A clearly shows the differences in EROD activity of the three types of endothelial cells after TCDD induction. There is a low level of activity in the endocardial and microvascular cells with DMSO alone. At the lowest concentration of TCDD (0.01 nM) the induced enzyme activity in the endocardial cells and the kidney microvascular cells is similar. EROD activity peaked at 1 nM TCDD in the endocardial cells and then decreases at 10 nM TCDD. The EROD activity in the kidney microvascular cells increased with increased concentrations of TCDD with the maximum activity at 0.3 nM TCDD but the activity (16.1 pmol/mg-min) was much lower than that obtained with the endocardial cells. Unlike the endocardial and microvascular cells, the capillary cells from the rete mirabile did not show expression of CYP1A activity with DMSO present. There was very little if any response from the capillary endothelial cells to TCDD or βNF. The EC_{50} for CYP1A induction by TCDD in heart endothelial cells is 0.013 nM and in the kidney cells is 0.01 nM.

Treatment of the endocardial cells with βNF induced maximum levels of CYP1A activity comparable to those obtained with TCDD induction (Fig 2 B). The concentrations of βNF were higher than those for TCDD (μM vs nM) to produce a

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comparable level of induction. The EROD activity peaked at 3μM βNF and decreased substantially at 10μM βNF.

<u>Immunochemical Identification of CYP1A</u>

Microsomes were prepared from heart endothelial cells induced with 0.3 μM βNF, 10 μM βNF and 1 nM TCDD for 48 hours in T-75 flasks. These preparations were chosen to confirm the presence of CYP1A in cells induced with both TCDD and βNF and that the level of CYP1A will reflect a high or low level of induction of EROD activity Fig. 3 shows an immunoblot of microsomal protein with the Mab 1-12-3 specific for CYP1A1 forms in fish (and CYP1A1 in mammals).

<u>Figure 3 here</u>

CYP1A level can be calculated based on the relative densities of the scup standards and the experimental blot. These are relative values since they are based on βNF induced scup liver microsomes with antibody Mab 1-12-3 developed against scup CYP1A. While the antibody reaction is specific for CYP1A the degree of antibody binding may not be as high with the eel CYP1A as it is for scup. The CYP1A content was calculated to be 18.5 pmol/mg with 1 nM TCDD induction and 1.41 pmol/mg with 10 μM βNF induction. The amount of CYP1A in the cells exposed to 0.3 μM βNF was below the level of detection with loading of 20.4 μg of microsomal protein. The immunoblots confirm that CYP1A is induced in the eel endothelial cells and the density of the blots reflect the level of CYP1A present with specific inducer protocols.

DISCUSSION

Endothelial cells cultured from the vasculature of the eel *Anguilla rostrata*
respond to the AhR agonists TCDD and βNF with the induction of CYP1A. This
induction is confirmed by increased EROD activity in intact cells and microsomes and
by immunodetection of CYP1A. However, the three types of endothelial cells respond
to induction differently. Induction of CYP1A is highest in the endocardial cells from
heart, lower in the microvascular cells from the kidney, with little or no induction in the
capillary cells from the rete mirabile. Thus, these cells can respond to AhR agonists in a
way that is specific for each inducer and also specific for each type of endothelial cell.
Are these cells a good model for what occurs *in vivo* in the endothelial cells?

In many *in vivo* experiments in teleosts and mammals, the endothelium in some
organs was more responsive than that in other organs to CYP1A induction by AhR
agonists, based on the expression of CYP1A detected immunohistochemically
(Stegeman et al., 1991; Stegeman et al., 1995). Schlezinger and Stegeman (2000a,
2000b) reported organ differences in CYP1A protein expression and activity (EROD) in
extra-hepatic organs of *Anguilla rostrata* after *in vivo* induction by xenobiotics and in a
natural population of eels exposed to high levels of PCBs (polychlorinated biphenyls).
The difference in the level of induction in the three endothelial cell populations parallels
the levels Schlezinger and Stegeman(2000a) reported for eel heart, kidney and rete
mirabile after *in vivo* induction by βNF. There is a consistent pattern of the strongest
EROD activity in the heart and only slight, if any, induction in the capillaries of the rete
mirabile and only with very high doses of inducer. The response in the kidney is
intermediate to the heart and rete mirabile. This same variation in the expression of

CYP1A was seen when organs were analyzed from eels collected from an environment contaminated with PCBs (Schlezinger and Stegeman, 2000b). Thus, the cells cultured from different types of vessels and from different organs in the eel respond differentially to induction by AhR agonists in a manner similar to that reported in intact animals. Another species of eel, *Anguilla japonica*, is reported to have two forms of CYP1A genes and in these genes there is more than one regulatory element that could play a role in gene expression (Ogino et al.,1999a; Ogino et al.,1999b). We do not have data that allow us to extend this information to interpret our results but the possibility of differential expression of two CYP1As is intriguing.

Until now mammalian endothelial cell cultures have been the cells used for studies of the cellular response in the vascular system to exposure to AhR agonists (Farin et al.,1994; Stegeman et al.,1995; Celander et al.,1997; Chat et al.,1998; Thum et al.,2000; Annas et al.,2000b; Savouret et al.,2001; Hennig et al.,2002a; Borlak et al.,2003; Ramadass et al.,2003). Although most of the mammalian endothelial cells have been cultured from large vessels differential responses to AHR agonists have been reported, for example, between human umbilical artery vs umbilical vein cells, (Annas et al.,2000b). The peak level of CYP1A1 activity (EROD) after TCDD or βNF induction in the mammalian large vessel endothelial cells was in the range of 3 to 30 pmol/mg-min (Stegeman et al.,1995; Celander et al.,1997; Annas et al.,2000b). This is considerably lower than the level we report for induction in the eel heart endocardial cells and about the same as we report for the kidney microvascular cells.

The EC_{50}, the inducer concentration that produces 50% of maximal EROD activity, is 0.013 nM TCDD for the heart endothelial cells and 0.01 nM TCDD for the

kidney endothelial cells. These values are similar to those reported for porcine aortic endothelial cells, 0.015 nM(Stegeman et al.,1995), transformed hepatocytes from fish, 0.016-0.022 nM (Hahn et al.,1996) trout gill, 0.04nM (Carlsson and Part,2001) and trout pituitary cells, 0.017-0.043 nM (Tom et al.,2001). In both the cultured endothelial cells (Table 1) and in organs taken from *Anguilla rostrata* (Schlezinger and Stegeman,2000a) there is a low level of EROD activity when there is no inducer of CYP1A present. Human aortic endothelial cells (HAEC), but not other mammalian endothelial cells, have EROD activity without exposure to CYP1A inducers (Celander et al.,1997).

We are not aware of studies that report CYP1A1 activity in intact cells with cultures of mammalian microvascular or capillary cells to compare to the data with the eel cells. Chat et al. (1998) reported a similar level of activity for P450 related enzymes in microsomes prepared from rat primary microvascular cerebrovascular endothelial cells (CVEC) and CVEC that had been immortalized (RBE4). A study that used pieces of tissue excised from the blood brain barrier of rats localized the induced expression of CYP1A to the cerebral veins and arteries, with the only activity in capillary endothelium in the vessels in the choroid plexus (Granberg et al.,2003) . The retention of *in vivo* expression of CYP isoforms in mammalian endothelial cells in culture was addressed in two recent reports. Thum et al. (2000) reported that when rat aortic endothelial cells de-differentiate there was a dramatic decrease in expression of all major CYP isoforms. (Chi et al.,2003) reported microarray analysis of 53 endothelial cell cultures that clearly demonstrated the differences in gene expression in arteries vs veins, large vessels vs microvascular vessels and in similar types of vessels in different organs. The results we report demonstrate that cultured eel endothelial cells, like the cultured mammalian cells,

retain the ability to respond in a selective manner to a specific stimulus such as an AhR agonist.

Cultures of other extra-hepatic cells from teleosts have been used to study CYP1A activity. Rainbow trout gill and pituitary cells show TCDD induced EROD activity of about 40 and 60 pmol/mg-min, respectively (Thum et al.,2000; Carlsson and Part,2001; Tom et al.,2001). We see that the level of EROD activity induced in the eel endocardial cells (113 pmol/mg-min, Fig 2) ranks high among extra-hepatic tissues from teleosts.

In summary: 1.) In heart endothelial cells the maximum response to TCDD, 113 pmol/mg-min, occurred at 1 nM TCDD and the peak response to βNF, 135 pmol/mg-min, at 3 μM βNF. 2.) In kidney endothelial cells the maximum response to TCDD was 12 pmol/mg-min at 0.3 nM TCDD. 3.) The rete mirabile capillary endothelial cells responded minimally or not at all to exposure to TCDD and βNF. 4.) Both the heart and kidney endothelial cells had a low level of EROD activity (12.7 and 5.2 pmol/mg-min respectively) in untreated or DMSO-treated cells. Insignificant activity (0 to 0.05 pmol/mg-min) was detected in untreated or DMSO-treated rete mirabile capillary cells.

The robust response of the heart endothelial cells to AhR agonists gives them the potential to be an excellent model to investigate the CYP response in vascular cells. This can be important given the implication of vascular effects in the toxicity of some AhR agonists. The lack of response in the capillary endothelial cells from the rete mirabile is also interesting in its specificity. The retention in culture of the differences in the separate populations of endothelial cells provides the potential to determine the

mechanisms of induction due to xenobiotics and to also explore the physiological

consequences of these events in different areas of the vasculature.

FIGURE LEGENDS

Figure 1. Endothelial cells isolated from the microvasculature of the kidney (A,B), the capillaries of the rete mirabile (C,D) and the endocardium of the heart (E,F) of the eel *Anguilla rostrata*. B,D and F are phase contrast micrographs and A,C and E are fluorescent micrographs showing the uptake of DiI-acetylated-LDL viewed on a Zeiss Axiovert S100 at 10X

Figure 2. (A) EROD activity induced by TCDD in cultured endothelial cells from the endocardium of the heart, microvasculature of the kidney and capillaries of the rete mirabile of the eel. (B) TCDD and βNF induction in endocardial cells. Note that TCDD values are in nM and βNF values are in μM. Values are mean $\pm$ SE of all the values for a given inducer concentration as listed in Table 1. The EC_{50} s calculated for the heart and the kidney endothelial cells for TCDD are 0.013 and 0.01 nM respectively.

Figure 3. Immunodetection of CYP1A in heart endocardial cells induced for 48 hours as described in the methods. (A) Scup control: (B) 0.3 μM βNF, 20.4 μg protein: (C) 10 μM βNF, 33.6 μg protein: (D) 1 nM TCDD, 25 μg protein. The CYP1A equivalents were calculated by comparison to scup βNF - induced liver microsomal preparation with a range from 0.1 to 0.4 pmol/mg of CYP1A.
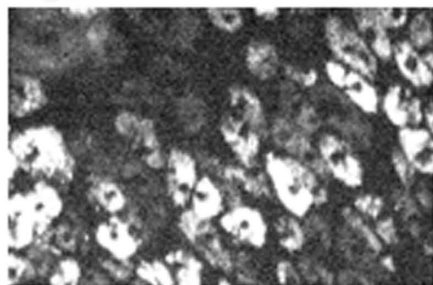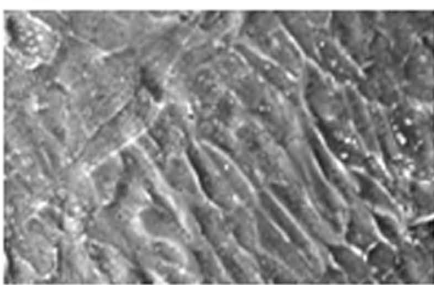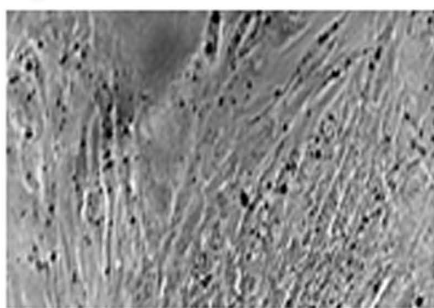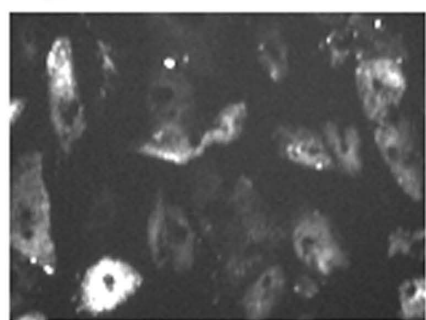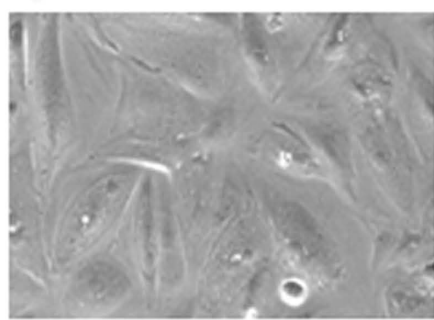
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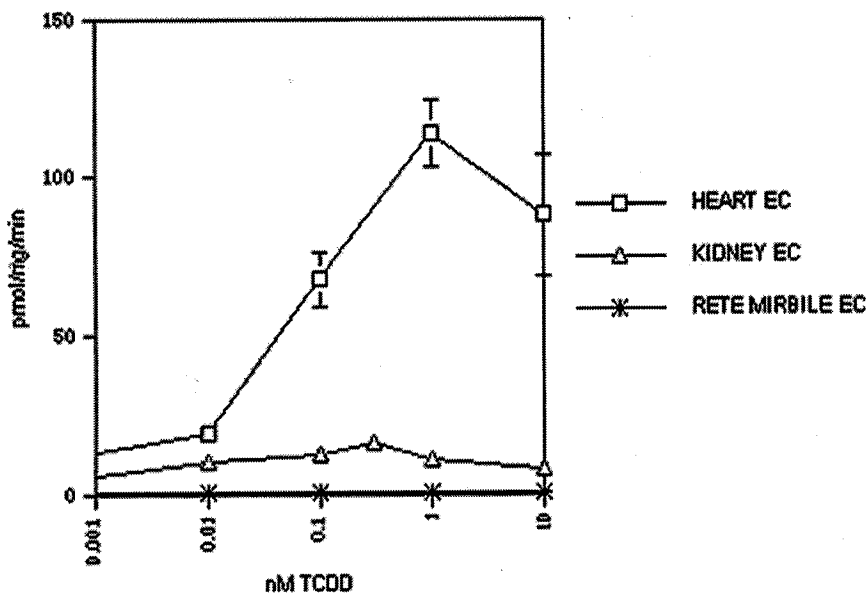
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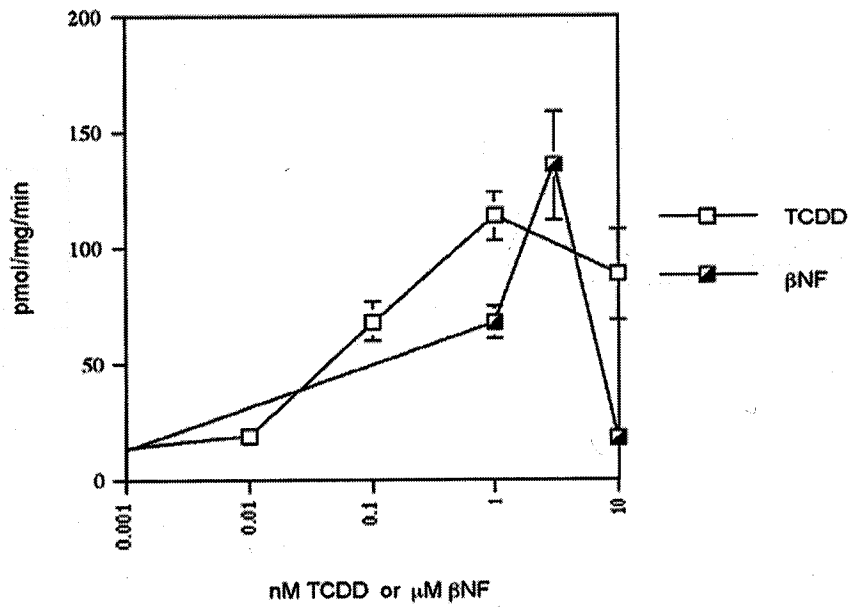
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A

B

C

D

E

F

Garrick,R.A.   Figure 1

A. TCDD INDUCTION IN EEL ENDOTHELIAL CELLS

HEART EC
KIDNEY EC
RETE MIRBILE EC

nM TCDD

B. TCDD or βNF INDUCTION in HEART EC

TCDD
βNF

nM TCDD or µM βNF

RA Garrick   Fig 2

A   B   C   D

Garrick, R.A.   Figure 3

Table 1
Production of resorufin from 7- ethoxyresorufin in endothelial cells (EC)
Cells are induced in 48 well plates for 48 hours by TCDD or βNF.

Each row is the result of a separate plate, each value is n = 6 except as noted with *.

ND=not detectable   Values are mean $\pm$ SE

EC source

nM TCDD	0	0.01	0.1	0.3	1	10	30
			pmol/mg/min				
Heart	10.8 ± 1.6	18.8 ± 2.1	70.0 ± 6.4		84 ± 8.4	43.9 ± 12.9	
	12.1 ± 3.9		65.8 ± 16		142 ± 8.8	132 ± 28	
Kidney	5.1 ± 0.33	10.1 ± 0.87	12.2 ± 0.64	16.1 ± 1.7	11.0 ± 0.84	8.1 ± 0.88	
Rete mirabile	*0.05	0.06	0.01		0.08	0.59	0.3
	ND	ND	ND	ND	ND	4.4 + 7.6	
	ND	ND			ND	ND	

μM βNF	0	1	3	10
		pmol/mg/min		
Heart	10.8 ± 1.6			24.3 ± 4.9
	12.1 ± 3.9	67.5 ± 6.7	135.3 ± 23.5	17.9 ± 4.9
Rete mirabile	ND	ND		ND