0026-895X/89/050723-07$02.00/0

MOLECULAR PHARMACOLOGY, 36:723-729

Cytochrome P450IA1 Induction and Localization in Endothelium of Vertebrate (Teleost) Heart

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Received April 12, 1989; Accepted August 23, 1989

SUMMARY

Previous studies have shown that high levels of cytochrome P450 can occur in cardiac microsomes of vertebrates [*Mol. Pharmacol.* **21**:517–526, (1982)]. Here we identify the dominant cardiac P450 in the marine fish scup as P450E, a teleost representative of P450IA1, and we describe its restricted cellular localization in the heart. Treatment of scup with β-naphthoflavone produced an unusually strong (10-fold) induction of spectrally measured P450 in cardiac microsomes, with specific content reaching levels (0.5 nmol/mg) similar to those induced in scup liver. Microsomal ethoxyresorufin *O*-deethylase and aryl hydrocarbon hydroxylase activities, catalytic functions of scup P450E, were induced in parallel with P450 content. Similar induction was seen in both atrium and ventricle. Immunoblot analysis with monoclonal antibody 1-12-3, specific to scup P450E and other vertebrate P450IA1 proteins, showed that this hydrocarbon-inducible P450 is the dominant and possibly sole P450 form in heart microsomes of experimentally induced animals. Immunohistochemical analysis of scup heart sections (2–4-μm) with monoclonal antibody 1-12-3 revealed that P450E was detectable only in endothelial cells of the endocardium and of the coronary vasculature. A similar endothelial cell localization of the monoclonal antibody 1-12-3 epitope was observed in heart of rainbow trout, induced with β-naphthoflavone, indicating a general nature for the endothelial localization of induced cardiac P450. Morphometric analysis showed that endothelium could constitute 8–9% of the volume of teleost heart, from which we calculate that P450IA1 could account for as much as 25% of the endothelial cell microsomal protein. Heart microsomes of untreated animals from contaminated environments also contained high levels of P450E, indicating that induction like that caused by β-naphthoflavone could occur with chemicals in the environment. Strongly induced P450E (P450IA1) in endothelium could play a critical role in chemical-biological interactions involving xenobiotics affecting the vasculature of the heart or other organs.

P450 catalyzes activation or inactivation of foreign compounds, including many drugs and carcinogens, and the synthesis or breakdown of endogenous compounds such as steroids (1). Numerous forms of P450 with distinct structural and catalytic properties have been isolated from vertebrates, principally from liver microsomes (2, 3). Compared with the levels of P450 in liver microsomes, the specific content of total microsomal P450 is low in most extrahepatic organs. However, previous studies have revealed high levels of P450 in heart microsomes of phylogenetically diverse vertebrates (4, 5), occurring in both atrium and ventricle of fish (6) and, more recently described, of mammals (7). The levels of P450 in heart approach and sometimes exceed those in liver (6). In spite of the high P450 levels, catalytic rates of cardiac microsomal monooxygenase activity reported with several substrates are comparatively low, in both fish and mammals (6, 7). It is possible that there are novel or unrecognized P450-dependent transformations of drugs or other toxicants that are preferred in heart. Understanding of the significance of this cardiac P450 will depend on establishing the identity of the P450 present and also the cell type(s) where it occurs. In these studies, the induction and distribution of cardiac P450 was evaluated in two teleost species by catalytic assay and by analysis with MAb 1-12-3 (8) to P450E isolated from the marine teleost scup (9). Based on structural, catalytic, and regulatory similarities (3, 9, 10), P450E is a teleost counterpart of rat P450c (BNF-B); i.e., they are orthologous proteins representing the BNF-, aromatic hydrocarbon-, and polychlorinated biphenyl-inducible P450IA1 (10, 11). MAb 1-12-3 specifically recognizes an epitope exclusive to P450IA1, which is conserved in all vertebrates examined to date, from fish to mammals (12). The results show that the aromatic hydrocarbon-inducible P450 is the dominant

This work was supported by United States Public Health Service Grants ES-4220 and CA-44306 to J.J.S. and CA-45131 to D.H. This is Contribution 6883 from the Woods Hole Oceanographic Institution.

ABBREVIATIONS: P450, cytochrome P450 monooxygenase; EROD, ethoxyresorufin *O*-deethylase; AHH, aryl hydrocarbon (benzo[a]pyrene) hydroxylase; BNF, β-naphthoflavone; MC, methylcholanthrene; TCDD, 2,3,7,8-tetrachloro-*p*-dioxin; anti-BrDU, monoclonal antibody to bromodeoxyuridine; MAb, monoclonal antibody; FITC, fluorescein isothiocyanate.

and possibly sole P450 form present in heart from treated animals. Furthermore, this P450 is induced primarily in the endothelial cells of the heart, which has important implications for cardiac physiology and for cardiotoxicity of chemical agents.

Materials and Methods

Reagents. JB-4 monomer was supplied by Polysciences, Inc. Ethoxyresorufin was synthesized as before (13). All other reagents were from Sigma Chemical Company. Scup liver cytochrome P450E was purified as previously described (9). MAbs, MAb 1-12-3 to scup P450E and anti-BrDU, were obtained as before (8, 14). FITC-conjugated goat anti-mouse heavy and light chains used as secondary antibodies were purchased from Cappell.

Animals and tissue preparation. Scup were obtained from local Woods Hole waters and were housed in facilities at the Environmental Systems Laboratory on a 12 hr/12 hr light/dark cycle. Fish were fed a diet of Purina Trout Chow. Experimental animals were held for a period of 12 months before treatment. This holding has been found to greatly reduce effects of environmental chemicals on P450, known to occur in these wild fish (12). Trout were obtained from the Bowden Federal Fish Hatchery (Bowden, WV) and were housed in the animal quarters at West Virginia University Medical School, as previously described (15). Scup were injected intraperitoneally with BNF, suspended in corn oil (20 mg/kg), on days 0 and 3 and were sacrificed on day 6. Control scup were injected with corn oil alone. Trout were injected intraperitoneally with BNF (50 mg/kg) suspended in corn oil, or with corn oil alone, on day 0 and sacrificed on day 3. These treatments are known to be effective in inducing P450E in liver of scup or the P450E homologue in trout. Scup were also taken directly from the environment and tissues were sampled and prepared within 10 hr of capture. These fish were taken at sites previously described (16), where they appear to be induced by environmental chemicals.

Animals were anaesthetized on ice and killed by cervical section. Pericardial cavities were rapidly opened and whole hearts, including atrium and ventricle, were removed. Slices (2 × 2 × 4 mm) through both chambers were oriented sagittally and ventricles were also cut transversely to yield tissue from the strata compacta and spongiosa. Slices of heart tissue were quick-frozen in liquid nitrogen or in precooled isopentane (−170°) and were stored at −60° until processed for immunohistochemistry as below. Other portions were used for microsome preparation. Liver tissue was also excised for microsome preparation.

Microsome preparation and analysis. Portions of whole scup heart, or separated atria and ventricles, were pooled and microsomes were prepared from this fresh tissue. Microsomal samples were from portions of the same hearts examined histochemically. Liver microsomes were also prepared from these animals. Microsomal fractions of both organs were obtained according to methods previously described (6). Cardiac and hepatic microsomes were also obtained from freshly caught scup. Cardiac microsomes of some of these wild fish were the same ones originally analyzed in a previous study (6). These microsomes had been archived in liquid nitrogen since that time. We find that scup microsomal P450E is stable for a period of years under these conditions.

Cardiac and hepatic microsomal cytochrome P450 content and cytochrome b_5 content and NADPH cytochrome *c* (P450) reductase activity were analyzed as before (6). EROD activity was analyzed spectrophotometrically and AHH activity was analyzed radiometrically according to methods previously described in detail (6, 13). Microsomal protein was analyzed according to the method of Lowry *et al.* (17).

Immunoblotting. Immunoblot (Western blot) analysis of control and treated cardiac microsomes was carried out with proteins separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose as previously described (12). Nitrocellulose was exposed to MAb 1-12-3 to P450E (8) (1:500), followed by horseradish peroxidase-linked goat anti-mouse IgG (Bio-Rad). Other details have been published (12). Immunoblots of whole heart microsomes were scanned with a laser densitometer (Helena Labs), and P450E content was calculated based on comparison with reaction obtained with purified P450E standards that were blotted together with the cardiac samples.

Immunohistochemistry. Frozen scup and trout heart slices obtained from BNF-treated and from control animals were dried under vacuum at −40% for 8–12 hr, placed in 100% catalyzed JB-4 monomer, and infiltrated under vacuum for 18 hr. The tissues were embedded in complete JB-4 glycol methacrylate and 2–4-μm sections were cut with a LKB Historange microtome. Sections were placed on glass slides, air-dried, and processed for hemotoxylin and eosin staining. For immunohistochemical studies, sections were exposed to either the P450E-specific MAb 1-12-3 or control primary antibodies for 1 hr at 25°. Control antibodies were mouse anti-BrDU previously described (14). Stock antibody solutions were ascites fluids containing 40 mg of protein/ml; these antibody solutions were diluted 100-fold in phosphate-buffered saline containing 1% bovine serum albumin before application to the sections. A range of antibody dilutions was treated for optimal staining. In some studies, 25 μl of 100-fold-diluted antibody solutions were pretreated with 2 μg of purified scup P450E at 2° for 45 min before application to sections. This procedure was to ensure that the immunohistochemical reaction was specific for cytochrome P450E in scup or its counterpart in trout. Sections were rinsed three times for a total of 15 min, and then incubated for 30 min at 37° with FITC-labeled second antibody that was diluted 20-fold in phosphate-buffered saline containing 1% bovine serum albumin. Following extensive rinsing, the sections were mounted with Fluoromount G and viewed on an Olympus BH-2 fluorescence microscope, fluorescence was scored, and sections were photographed as before (15).

Immunological staining within a set of slides was rated subjectively, on a scale of 0 to +3, by separate investigators who did not know the origin of the tissue, or the antibody treatment. Following subjective scoring, the most brightly fluorescent fields observed were photographed using the automatic exposure meter. Intertreatment comparisons of intensity of fluorescence were possible by timing this exposure and manually exposing all subsequent fields for identical periods of time. Photographs of these fields were obtained under identical conditions.

Results

P450 induction in heart. The specific content of total P450 in scup whole heart microsomes was induced 8- to 10-fold by BNF in separate experiments. The levels of P450 induced were as high as those in liver of the same BNF-treated fish (Table 1). Liver microsomal P450 content in these same animals increased by about 40%. The Fe^{2+}-CO absorption maximum of heart microsomes from control and treated animals alike was 447 nm. EROD and AHH specific activities, catalytic functions of P450E (9), were also induced by BNF in whole heart microsomes (Table 1). In contrast to the content of induced P450, however, these catalytic rates induced in heart microsomes were substantially lower than the rates of these activities induced in liver microsomes of the same animals (Table 1). The magnitude of induction of catalytic activity/mg of protein in heart was comparable to the extent of change in P450 content. Accordingly, the estimated turnover number (activity/nmol of total P450) in heart microsomes was not increased by BNF. In liver the estimated turnover number was increased by BNF.

In repeated experiments, the specific content of total P450 was again strongly induced (nearly 10-fold), to levels like those in liver, in microsomes prepared separately from either scup atrium or ventricle. The Fe^{2+}-CO absorption maximum was 447

TABLE 1

Induction of microsomal cytochrome P450 in scup heart and liver

Scup were treated intraperitoneally with corn oil (control) or 20 mg/kg BNF in corn oil. The results shown here are for one experiment. Heart tissue from six animals was pooled. Liver microsomes from these same animals were prepared and analyzed separately.

	Heart		Liver	
	Control	BNF	Control	BNF
Microsomal protein (mg/g of organ)	3.5	1.4[a]	9.0 ± 3	12.0 ± 3
P450 (nmol/mg)	0.06	0.50	0.33 ± 0.04	0.55 ± 0.16
Fe^{2+}-CO λ_{max} (nm)	447	447	450	449
EROD				
nmol/min/mg	0.033	0.235	1.12 ± 0.37	5.12 ± 1.61
nmol/min/nmol of P450	0.49	0.47	3.5 ± 1.3	9.5 ± 1.5
AHH				
nmol/min/mg	0.116	0.688		
nmol/min/nmol P450	1.73	1.37		

[a] The basis for a lower heart microsomal protein yield in treated animals is not known but has been observed repeatedly. Similar levels of cardiac and liver microsomal P450 were obtained in repeated experiments. The content of P450/g of organ was still induced 3-fold in heart.

nm in microsomes from both parts of the heart. EROD and AHH specific activities were also induced nearly 10-fold in both atrium and ventricle but, as in whole heart, the rates of activity were lower than those in liver and there was little change in turnover number in cardiac samples. Levels of NADPH-cytochrome *c* (P450) reductase activity were about 8 nmol/min/mg in atrium or ventricle, an activity equal to less than 10% of the activity commonly seen in scup liver microsomes, about 110 nmol/min/mg.

Immunoblot (Western blot) analysis with MAb 1-12-3 to scup P450E showed a single cross-reacting protein in BNF-induced cardiac tissues, coincident in migration with authentic P450E (Fig. 1). Given the specificity of MAb 1-12-3, these immunochemical results confirm induction of P450E in heart. Calculating the specific content of P450E reveals that this single form could constitute 100% of the total microsomal P450 in heart of BNF-treated animals (Table 2). There was a faint but unquantifiable band in control microsomes.

The above results show a strong experimental induction of P450E in heart. Analysis of cardiac microsomal P450E content in animals taken directly from the environment also showed high levels of P450E. The content of P450E detected upon immunoblot analysis of heart microsomes from these scup was the same order of magnitude as that seen in BNF-treated fish (Table 2). It should be noted that, based on the levels of EROD activity, liver P450E was also strongly induced in these same wild fish (6).

Localization of cardiac P450E. Evaluating the cellular localization of cardiac P450E showed that it was present primarily in one cell type. The cellular structure of scup ventricle (Fig. 2A) shows the endocardial endothelium bordering the chamber lumen and the bundles of cardiac muscle in a region of bundle bifurcation termed the stratum spongiosum, a common feature in teleost heart. Sections of ventricle from BNF-treated scup incubated with MAb 1-12-3 and FITC-labeled second antibody showed strong fluorescence that was restricted to the endothelium (Fig. 2B). Similar sections from other BNF-treated scup were examined at higher magnification. These sections further show that the intense fluorescence in BNF-treated fish was strongly localized in endothelial cells (Fig. 2C).

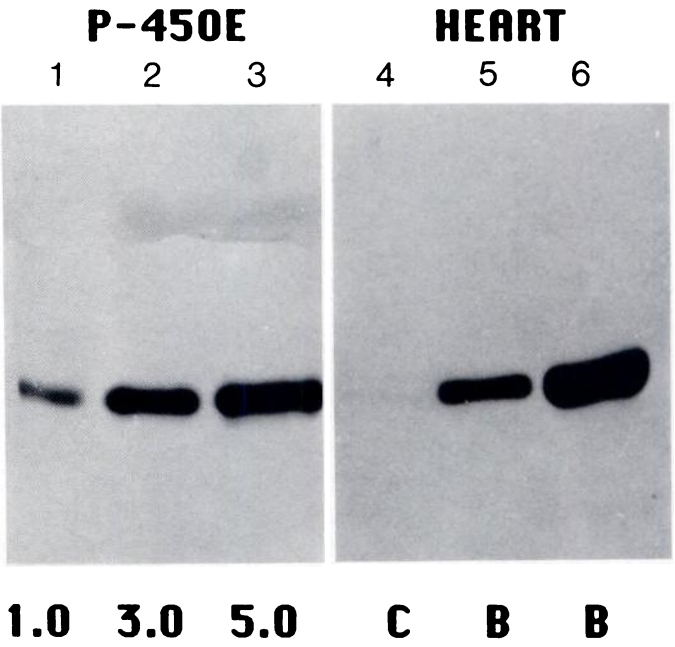

Fig. 1. Immunoblot of scup heart microsomes stained with MAb 1-12-3 to P450E. *The Lanes 1–3* contained 1.0, 3.0, or 5.0 pmol of authentic P450E. *C*, control; *B*, BNF-treated fish. *Lanes 4* and *5* contained similar amounts of microsomal P450, and *lanes 4* and *6* contained similar amounts of microsomal protein. *Lane 4* contained approximately 2 pmol of heart microsomal P450 from control fish; *lane 5*, 2 pmol of heart microsomal P450 from BNF-treated fish; *lane 6*, 15 pmol of heart microsomal P450 from BNF-treated fish. *Lanes 4* and *6* each contained 40 μg of microsomal protein.

TABLE 2

Quantitation of P450E in cardiac microsomes

Treatment	*n*[a]	Heart tissue	P450E content[b]	Total P450[b]	P450E content
			nmol/mg	*nmol/mg*	*% of total*
Experimental					
Control (corn oil)	6	Whole heart	<0.01[c]	0.05	~15
BNF	6	Whole heart	0.52	0.50	~100
Environmental[d]					
Sample 1	25;2	Whole heart	0.13	—[e]	
Sample 2	22	Atrium	0.29	0.25	~100

[a] *n*, number of fish from which heart tissue was pooled. 25;2 refers to 25 fish in two pooled samples.

[b] P450E content refers to P450E quantitated in immunoblots, as in Fig. 1. Total P450 refers to spectral determination of P450 in microsomes.

[c] Not detectable. The detection limit was approximately 0.01 nmol/mg.

[d] Environmental samples were taken at the mouth of the Woods Hole Channel. Value for whole heart is average of two preparations; range is ±0.033. The atrium sample is the same sample described previously (6); EROD activity in liver microsomes from those same fish was 3.7 nmol/mg/mg.

[e] Spectral P450 content not measured.

Ventricular and atrial sections were studied and no differences were found in immunohistochemical staining of these two portions of the heart. The P450E detected with MAb 1-12-3 was found primarily in the endothelial cells of all animals examined; no specific reaction was detectable in cardiac myocytes. Furthermore, presorbing MAb 1-12-3 with highly purified P450E in all cases abolished the reaction in induced heart sections. There was likewise no reaction in sections of BNF-treated scup heart that were incubated with control primary antibody, anti-BrDu.

The general nature of this cellular distribution of BNF-induced P450 in heart was established by examining another species, rainbow trout. MAb 1-12-3 cross-reacts specifically with the BNF- or polyaromatic hydrocarbon-inducible P450

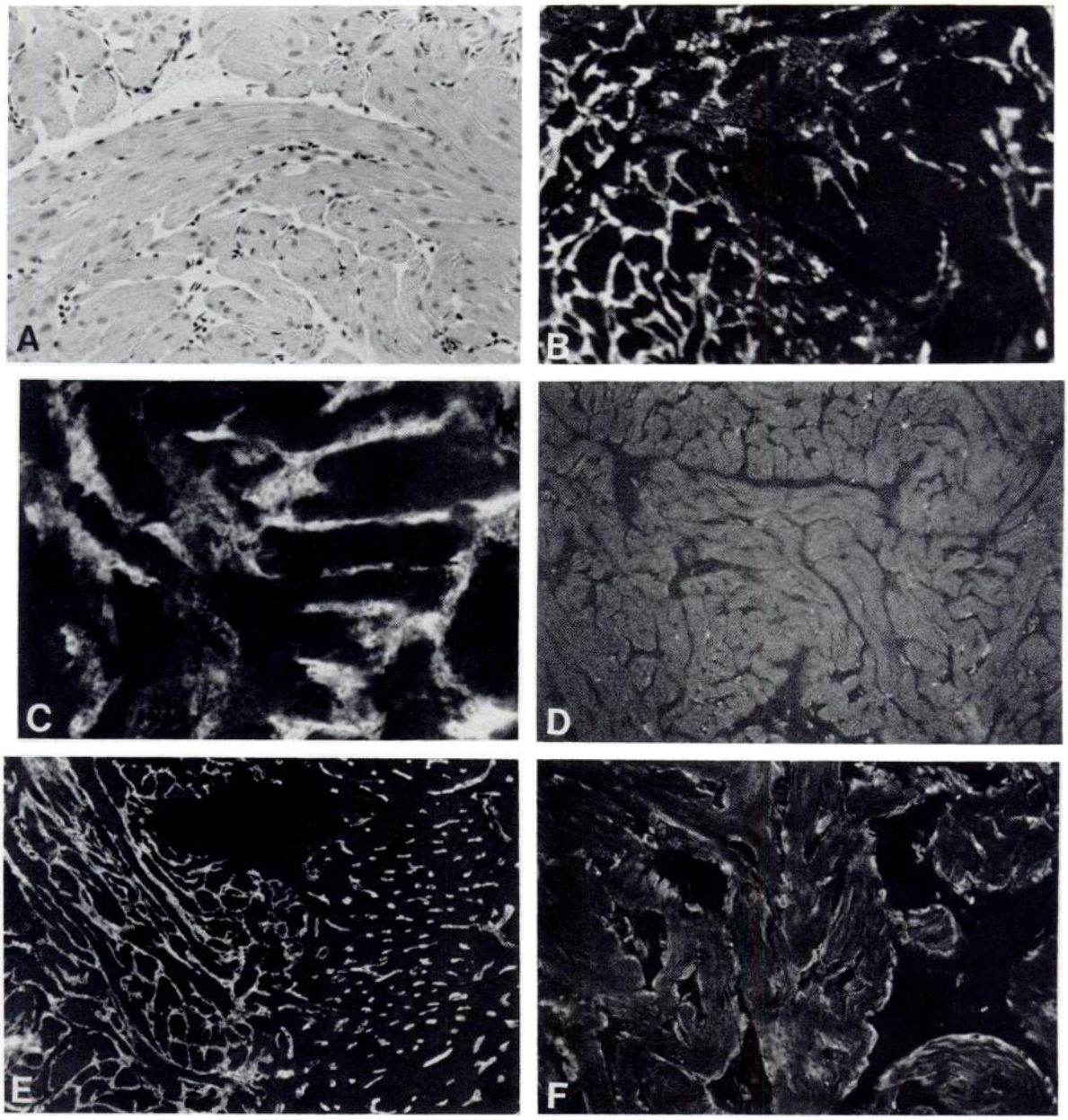

Fig. 2. A, Hematoxylin- and eosin-stained section of scup heart showing atrium (*top*) and strata compactum (*middle*) and spongiosum (*bottom*) of ventricle. B, BNF-treated scup ventricle reacted with MAb to P450E showing broad field (original magnification, 75X). C, Section of BNF-treated scup ventricle reacted with MAb 1-12-3. Note strongly fluorescent elongate cells (endothelium) and endothelial cell nucleii (original magnification, 130X). D, BNF-induced rainbow trout ventricle reacted with MAb 1-12-3 that had been presorbed with cytochrome P450E, followed by fluorescent second antibody. All sections of scup or trout heart that were reacted with presorbed 1-12-3 or with anti-BrDU gave only negative results or background staining. E, BNF-induced rainbow trout ventricle reacted with MAb 1-12-3 and FITC-labeled second antibody (original magnification, 75X). Note similarity to B. F, Control rainbow trout ventricle incubated with MAb 1-12-3. Slight fluorescence appears at edges but there is little or no staining of cells.

(P450IA1) from other vertebrates, including that from trout (12). As with control scup, sections of control trout heart showed MAb 1-12-3 reaction only at edges (Fig. 2F), with little specific staining of endothelium. However, in heart preparations from BNF-treated trout, MAb 1-12-3 strongly detected a protein only in endothelial cells (Fig. 2E). This is more clearly seen in Fig. 3. As with scup, presorption of MAb 1-12-3 with P450E abolished the staining of trout endothelium and BNF-treated trout heart sections treated with anti-BrDU showed no staining (Fig. 2D).

The sections in Figs. 2 and 3 show localization in endothelial cells that are components of the endocardium. In other sections of induced scup and trout heart, P450E was also detected in the endothelial cells lining coronary arteries of the stratum compactum, the outer region of the ventricle, but not in other cells.

The intensity of fluorescence in heart sections from numerous individuals of scup and trout that were control- or BNF-treated was examined under identical micrographic conditions in a blind test with two investigators. This analysis repeatedly and reproducibly showed greater amounts of P450E in endo-

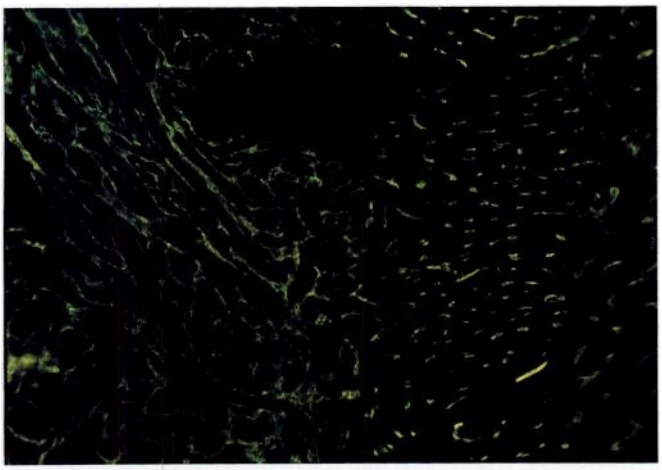

Fig. 3. Color enlargement of Fig. 2E, BNF-induced trout ventricle reacted with MAb 1-12-3.

TABLE 3

Relative intensity of immunostaining of cardiac endothelial cells

Companion sections were treated with anti-BrDU or with MAb 1-12-3. Relative intensity was determined by scoring in blind trials as described in Materials and Methods. Studies with three or more fish in each group gave identical results.

Sample treatment	Primary antibody	Relative intensity
Scup		
Control	Anti-BrDU	0
	MAb 1-12-3	0 to +*
BNF	Anti-BrDU	0
	MAb 1-12-3	+++
Trout		
Control	Anti-BrDU	0
	MAb 1-12-3	0 to +
BNF	Anti-BrDU	0
	MAb 1-12-3	+++

* Some control fish were scored as 0 and some as +.

thelium of heart from BNF-treated animals than from control animals, for both scup and trout (Table 3).

Because P450E occurs primarily in the endothelium and the protein applied to immunoblots would have contributions from endothelial and nonendothelial cells, we sought to estimate the amount of endothelium that might contribute to cardiac tissue microsomes. Estimation of the cellular composition of the heart was done by point count morphometry. A grid, with interlacing lines equidistant from one another, was positioned over photographs of immunohistochemical reactions in hearts of scup and trout. Assuming the fluorescent areas to be endothelium, the number of points (intercepts) over endothelium, muscle, and heart lumina were determined. Points over lumina were subtracted from total points (468) and a ratio of points over endothelium to points over total tissue (endothelium + muscle) was established. Such areal ratios have been shown to be equivalent to volume rations (18). Because fluorescence was our marker for endothelium, estimates of heart tissue volume occupied by endothelium differed between control and BNF-treated fish. For control heart tissue, the volume that was occupied by endothelium was about 9.5% in scup and about 8.4% in trout.

Discussion

The induction of total P450 content and of EROD and AHH activities demonstrates a strong response to BNF in teleost heart. Furthermore, the levels of P450E detected immunochemically indicate that the response to BNF involves primarily this P450IA1 protein. In a previous study, treatment of scup with MC produced little change in levels of cardiac microsomal P450 or in levels of monooxygenase activity (6). The distinction between that result with MC and the present results with BNF could reflect a preinduction by environmental chemicals in the fish in the previous study, a condition known to occur in some of these animals (12). The characteristics of "control" heart microsomes in that earlier study resemble those of BNF-treated animals here. For example, the Fe^{2+}-CO absorption maximum of heart microsomes was the same, 447 nm, in both studies. This value, which also characterizes purified P450E, has been consistently seen in scup heart microsomes, including control samples here. The spectral properties and immunohistochemical results suggest that low levels of P450E were present in control hearts here, further indicated by immunoblot analysis. The detection of high levels of P450E in some of the earlier untreated heart preparations by immunoblot analysis of those same microsomes in the present study (see Table 2) confirms that some untreated fish are partially induced.

The content of P450E in BNF-induced cardiac microsomes, 0.50 nmol/mg, and its strong localization in endothelium indicate that the concentration of P450E in endothelial cells could be very high. Using standard point count morphometric analysis (18), we estimated that the endothelial cells contribute about 8–9% of the heart cellular mass in both scup and trout. If these cells contribute to scup heart microsomes in that proportion, then the P450E content in BNF-treated heart endothelium could range up to 5 nmol/mg of endothelial cell microsomal protein or greater. Given its estimated molecular weight near 54,000 (9), this single P450 form could amount to nearly 25% of cardiac endothelial cell microsomal protein in highly induced samples.

Guengerich and Mason (5) observed that ethoxycoumarin *O*-deethylase activity induced in rat heart microsomes by MC could be inhibited by polyclonal antibodies to rat P450 MC-B (P450IA1). They also noted that the absorption maximum of rat heart microsomes was 447 nm, which, as in scup, is lower than the Fe^{2+}-CO λ_{max} of 448–450 nm seen in microsomes of other organs (5, 6). The absorption maximum of pure rat P450IA1 is 447 nm. The spectral and catalytic data suggest that induction of P450IA1 may be a common feature in heart of diverse vertebrate groups. Whether the strength of induction

in mammals might be similar to that in lower vertebrates, where it appears to account for nearly all the P450 present in heart of BNF-treated animals, is unknown. The catalytic rates in cardiac microsomes of induced mammals or fish are much lower than might be expected based on the level of P450 evident (5, 7). The low EROD and AHH turnover numbers in control and induced teleost heart microsomes could occur if the catalyst for these activities had a similar relative abundance in both groups and if some factor was limiting the activity. The levels of P450 reductase activity in induced scup heart correspond to a molar ratio of P450 to reductase of about 200:1, nearly 10 times the 20:1 ratio in scup liver (9). Serabjit-Singh *et al.* (19) did not detect staining of lung vascular endothelium by antibodies to P450 reductase, suggesting that although P450 is present in endothelium it might not participate in monooxygenase functions or that other electron-transfer mechanisms may be involved. Our observation of inducible EROD activity, a function of P450E, suggests that the endothelial P450E does participate in monooxygenase reactions. We have also found that polyclonal antibodies to P450E (12) at 5 mg IgG/mg of microsomal protein (~30 mg/nmol of P450) are able to inhibit cardiac microsomal EROD activity to the same degree that they inhibit liver microsomal EROD activity (data not shown). This indicates that P450E is functioning in cardiac microsomes, but the presence of reductase and its coupling to P450E in endothelial cells remains to be explicitly shown.

This is the first evidence showing that the major BNF-inducible P450 (P450IA1) is the dominant and possibly sole form present in the heart of some induced vertebrates and that it is strongly localized in the endothelium. P450 has been detected in vascular endothelial cells of some other organs, in both fish and mammals. We have seen P450E induction in fish gill endothelium (15), and rabbit P450 6 (a P450IA1 orthologue of P450E) and P450 4 (P450IA2) were seen in endothelium of lung and kidney of rabbits treated with TCDD (20). Induction of P450 in endothelial cells of mouse lung and gut by MC was more recently detected (20) with MAb 1-7-1 to rat P450c (P450IA1). The magnitude of endothelial cell induction of specific P450IA forms in mammals has not been determined; in many mammalian organs the P450 forms may be induced in cell types additional to endothelium (e.g., Ref. 21). In rabbit aorta most if not all P450 6 may be in smooth muscle rather than endothelium (22). Nevertheless, the appearance of BNF-, TCDD-, or MC-induced P450s in endothelium of different organs of diverse vertebrates establishes this as a general response in this cell type. It also implies that similar regulatory mechanisms for P450IA genes are operating in endothelium of diverse organs. Based on the above and on the strength of induction in teleost heart, endothelium must be considered a possible primary site for extrahepatic P450IA1 induction by aromatic hydrocarbons, polychlorinated biphenyls, dioxins, or dibenzofurans. In this regard we have, in preliminary studies, observed induction of P450IA1 by tetrachlorodibenzofuran in endothelium of all organs examined, including heart and brain[1].

Other forms of P450, i.e., rabbit forms 2 (P450IIB1) and 5, have been unequivocally detected in endothelium of rabbit lung (19). However, recent studies failed to detect P450IIB1 mRNA or protein in rat heart, including that from animals treated with phenobarbital (23). Whether P450 forms other than those representing the P450IA gene family could contribute to endothelial cell monooxygenase functions in heart, particularly uninduced heart, needs to be determined. In this regard, the faint P450E band in control heart (Fig. 1) could indicate that the 2 pmol/mg P450 includes forms other than P450E. On the other hand, the faint band might indicate that the total amount of P450 in those control samples was less than we had estimated spectrally.

High levels of P450E or its counterparts induced in cardiac endothelium could be adaptive, protecting the organ from effects of some blood-borne xenobiotics. Other evidence indicates a role for endothelium as a barrier of defense, protecting the heart from chemical and/or biological insult (24). However, the *in vitro* rates of P450E catalytic activities (EROD and AHH) were lower than expected based on the levels of P450E detected. It is possible that intracellular rates might be higher and that there might be undescribed catalytic functions of P450E (perhaps with cardioactive natural products) somehow favored in this cell system. However, the low activities also suggest that xenobiotic parent compounds that would be substrates for P450E might readily pass to the cardiac myocytes.

In contrast to the potential for P450E induction to serve a protective role, there could be enhanced activation of foreign compounds in the endothelium. P450E and other P450IA1 forms are primary catalysts forming the 7,8-dihydrodiol of benzo(*a*)pyrene (9), a procarcinogenic derivative that we find as a major benzo(*a*)pyrene metabolite formed also by cardiac microsomes (6). Juchau *et al.* (25) hypothesized that activation of such promutagens by vascular tissue (aorta) P450 could initiate lesions leading to atherosclerosis. Furthermore, metabolism of endogenous compounds essential to maintenance of vascular function, such as prostaglandins, could be altered by induction of this form of P450 in the endothelium. P450IA1 from rabbits catalyzes ω-2 hydroxylation of prostaglandin E (26), and in chick embryos BNF treatment apparently alters cardiac prostaglandin metabolism (27). Other studies have implicated P450 in vasoactive processes (28). High levels of P450 in endothelium might also bind nitric oxide, an apparent endothelium-derived relaxing factor (29) and a ligand for P450.

Cardiac and cardiovascular toxicity of compounds including TCDD have been reported (30, 31). The possible interactions of induced P450 with exogenous and endogenous substrates or ligands could be highly significant in the response of the cardiovascular system to such compounds in the environment. As discussed above, the characteristics of heart microsomes from untreated fish in an earlier study and the immunoblot results obtained with those same samples here show that induction of cardiac P450E could be caused by levels of chemicals present in the environment. Clearly the potential for induced endothelial P450 to participate in toxic interactions in the heart deserves attention. Teleost heart, which has cardiac muscle bundles bordered by abundant endothelium, could serve as a model for investigating the mechanism(s) for the strong induction and the biological significance of P450IA1 in cardiac endothelium.

Acknowledgments

We gratefully acknowledge the technical assistance of Bruce R. Woodin, Francis Y. Teng, and Swee Teh. We thank Drs. S. S. Park and H. V. Gelboin for continuing and generous collaboration.

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