We have examined the expression of cytochrome P-450IA1 (Cyp1a1) in cultured rat arterial smooth muscle cells (SMC). We report here that newborn (pup) aortic and adult carotid neointimal SMC express 10–20-fold higher steady-state levels of Cyp1a1 mRNA compared with identically treated SMC derived from adult aorta or uninjured carotid media, respectively. Nuclear transcription experiments suggest that the differences in basal expression of Cyp1a1 reflects differences in transcription rates of the gene in these two cell types. Treatment of adult aortic SMC with cycloheximide dramatically elevated Cyp1a1 mRNA levels and had little effect on the amount of this mRNA in pup SMC. The cycloheximide effect in adult SMC was due to enhanced transcription of the Cyp1a1 gene and was demonstrated by 1) the ability of actinomycin D to completely block the cycloheximide-induced increase in steady-state mRNA levels, and 2) the increased transcription rate of the Cyp1a1 gene in cycloheximide-treated adult nuclei determined in nuclear transcription experiments. Treatment of either cell type with β-naphthoflavone increased Cyp1a1 mRNA levels to a similar extent, suggesting that the differential response to cycloheximide was specific and not a general lack of pup SMC to respond to transcriptional inducers of the Cyp1a1 gene. Our data suggest that a labile repressor of the Cyp1a1 gene is present in cultured adult aortic SMC and is absent, inactive, or at a greatly reduced level in cultured pup SMC.

It has been proposed that smooth muscle cell (SMC) proliferation in hypertension and atherosclerosis recapitulates events occurring during differentiation (1). In support of this hypothesis, we have observed that cultured SMC derived from newborn (pup) rat aortas display differences in morphology, growth rate, ploidy, and platelet-derived growth factor-B gene expression when compared with cells derived from adult animals (2–4). Interestingly, the pup-like phenotype appears to be re-expressed in SMC cultured from the neointima formed in response to balloon catheter injury (5). In efforts to understand factors which regulate smooth muscle cell differentiation, we have focused our initial attention on defining genes whose expression is differentially regulated between pup and adult SMC.

Studies of genes whose activities appear to be developmentally regulated in vascular SMC in vivo have focused on cytoskeletal proteins including α- and γ-actin, vimentin, desmin, α-tropomyosin, and myosin heavy chain isoform 2 (6–8). Concentrations of these proteins are low in fetal SMC and increase in adult SMC. However, SMC in culture undergo modulation to a less differentiated phenotype, resulting in low levels of expression of many of these cytocontractile proteins (1). We therefore sought to identify genes whose expression displayed large and stable differences between pup and adult SMC in vitro to allow detailed study of molecular mechanisms responsible for SMC stage-specific gene expression.

The present paper reports the observation that a member of the mixed function oxygenase family displays differential regulation in cultured SMC derived from rat pup and adult aorta. Cyp1a1 was chosen, in part, because of suggestions that alterations in mutagenicity may play a role in the formation of atherosclerotic lesions (9, 10). Cyp1a1 is a polyaromatic hydrocarbon-inducible monoxygenase well known for its ability to convert procarcinogenic and promutagenic agents such as dimethylbenzanthracene and benzo(a)pyrene to their reactive forms (11). This enzyme is thought to play a pivotal role in the genesis of chemically induced carcinogenesis (12). A potential role for Cyp1a1-mediated metabolism in cardiovascular processes has been suggested by studies demonstrating aryl hydrocarbon hydroxylase activity in the hog (13), rabbit, monkey, and human aorta (14), as well as human fetal SMC (15). Furthermore, Serabjit-Singh et al. (16) have localized rabbit cytochrome P-450 Form 6, analogous to the rat Cyp1a1 form, to aortic smooth muscle cells by immunoblotting and enzymatic activity (16). Our findings establish that vascular SMC derived from pup aorta constitutively express Cyp1a1 in vitro and that both pup and adult SMC are inducible by a common Cyp1a1-inducing agent, β-naphthoflavone. Furthermore, our data suggests that the ontogenic regulation of Cyp1a1 observed in SMC in vitro may be at the level of a labile, developmentally controlled repressor of Cyp1a1 gene transcription.

MATERIALS AND METHODS

Cell Culture and Treatment—Thoracic aortas from 12-days (pup) or 5-month-old (adult) male Wistar-Kyoto, Sprague-Dawley, or spontaneously hypertensive rats were removed and stripped of endothelium and adventitia. SMC were obtained by collagenase and elastase digestion as previously described (2). Adult carotid neointimal SMC were provided by Dr. M. Reidy (University of Washington) and were derived from 3-month-old Sprague-Dawley rats 2 weeks after balloon catheter injury as previously described (8). Carotid medial SMC were derived from the uninjured, contralateral carotid of the same animal. All cells showed positive reactivity with CGA7 (17), a monoclonal antibody recognizing α-smooth muscle actin, demonstrating their

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†To whom correspondence and reprint requests should be addressed: Dept. of Pathology, SJ-60, University of Washington, Seattle, WA 98195.

‡The abbreviations used are: SMC, smooth muscle cell; Cyp1a1, cytochrome P-450IA1.
smooth muscle origin (2, 5). All cells were grown under identical conditions in Waymouth's medium supplemented with 10% adult bovine serum (Hyclone), 100 units/ml penicillin, and 0.1 mg/ml streptomycin unless otherwise stated. Cycloheximide, β-naphthoflavone, and actinomycin D were purchased from Sigma. Human platelet-derived growth factor was kindly provided by Dr. Russell Ross. CM-Sephadex-absorbed plasma-derived serum as essentially nonmitogenic and was prepared exactly as previously described (18). All experiments were performed on cells at confluence.

RNA Isolation and Northern Blotting—Total RNA was isolated by the method of Chomczynski and Sacchi (19). Five to 20 μg of RNA were electrophoresed on 1.2% agarose gels containing 6% formaldehyde. RNA was transferred to Zetaprobe (Bio-Rad) or GeneScreenPlus (Du Pont-New England Nuclear) according to the manufacturer's direction. Blots were hybridized 16 h at 42 °C in buffer containing 50% formamide, 0.75 M NaCl, 50 mM Tris, pH 7.5, 1 × Denhardt's (0.02% Ficoll, 0.02% polyvinylpyrrolidone), 300, 0.02% bovine serum albumin, 1% sodium dodecyl sulfate, 10% dextran sulfate, 200 μg/ml salmon sperm DNA, and 0.5–1.0 × 10⁶ [32P]cDNA. Filters were washed 2 × 5 min at room temperature and 2 × 30 min at 65 °C in 2 × SSC (standard saline citrate), 0.1% sodium dodecyl sulfate, and 2 × 30 min at 65 °C in 0.3 × SSC, 0.1% sodium dodecyl sulfate. Autoradiography was performed for the specified exposure times using Kodak XAR-5 film and Du Pont Lightning Plus intensifying screens. Molecular sizes were estimated by comparison of bands to the migration of 18 S and 28 S ribosomal RNA markers.

Nuclei Isolation and Run-off Assays—Approximately 20 × 10⁶ cells were washed twice in ice-cold phosphate-buffered saline, sonicated in 15-ml Falcon 1026 tubes, and centrifuged 5 min at 1200 rpm. Cell pellets were resuspended in 2 ml of lysis buffer containing 10 mM Tris, pH 7.4, 10 mM NaCl, 3 mM MgCl₂, 0.5% Nonidet P-40, and 0.5 mM phenylmethylsulfonyl fluoride, and incubated 5 min on ice. Nuclei were collected by centrifugation at 500 × g for 5 min. Nuclei were washed once in lysis buffer, resuspended in 140 μl of 40% glycerol, 50 mM Tris, pH 8.3, 5 mM MgCl₂, and 0.1 mM EDTA, and used immediately in run-off assays. Nuclear run-off assays were performed essentially as described by McKnight and Palmiter (20) with the following modifications. Reactions were performed in 200 μl of buffer containing 150 μM of nuclei, 150 mM KCl, 1.5 mM MgCl₂, 100 mM ATP, CTP, and GTP, 2 mM creatine phosphate (Sigma), 35 μg/ml creatine phosphokinase (Sigma), 7 μg/ml nucleotide-diphosphokinase, 2.5 mM dithiothreitol, and 200 μl of [32P]UTP (Du Pont-New England Nuclear, 800 Ci/ml). Under these conditions, incorporation of [32P]UTP into RNA was linear for 50 min. [32P]RNA was hybridized to 5 μg of the appropriate cDNA or pUC slot-blotted onto nitrocellulose in small seal-a-meal bags. All hybridizations and washings were carried out as described (20). Filters were exposed for autoradiography 1–5 days, then each slot was carefully excised and radioactivity eluted with 250 μl of 0.04 M NaOH, neutralized with 100 μl of 1 M acetic acid, and counted 30–40 min in 6 ml of Aquasol. Specifically bound cpm's were determined by subtracting cpm's/min bound to a control vector, pUC or pGEM. Greater than 5 cpm bound after background subtraction was considered significant. Transcription rates in parts/million (ppm) were determined using the following equation, as described by Turcotte et al. (21): specific cpm's bound/[hybridization efficiency] × (fraction of 1 transcript length detected by probe) × (input [32P]-mRNA cpm's) × 10⁶ where hybridization efficiency was calculated as 0.25, 0.25 of 1 transcript length detected by probe for pc1 = 342 base pairs/6500 base pairs = 0.053, and input cpm's varied from 2 to 15 × 10⁶ cpm. Experiments were performed in duplicate and repeated independently at least twice.

DNA Probes—pc1 is a 341-base pair cDNA spanning nucleotides 1359 to 1699 of the rat Cypl mRNA. It was synthesized by oligo(dT)-primed reverse transcription of pup SMC total RNA followed by the polymerase chain reaction under conditions previously described (22), and cloned into pGEM. The polymerase chain reaction primers used to generate the fragment were 5'-CCATGACCCAGGACATTGATGGG-3' encoding nucleotides 1559–1378 and 5'-TCTGGTCGAGCATTCCAGACA-3' encoding nucleotides 1680–1699 of the rat Cyp1a mRNA (23). Authenticity of the clone was established by sequence analysis (data not shown) which indicated that the sequence of pc1 was identical to that reported for the Cyp1a mRNA isolated from rat liver (23). rif-1, a 500-base pair rat fibroblast cDNA, was kindly provided by Dr. Richard Hynes (Massachusetts Institute of Technology) (24). A 350-base pair cDNA for the mouse c-myc gene spanning exon 2 was a gift from Dr. David Morris (University of Washington) (25). A cDNA encoding 1.3 kilobases of the glyceraldehyde-3-phosphate dehydrogenase has previously been described (26). For Northern blots analyses, isolated cDNA inserts were radiolabeled using a Schleicher & Schuell multiprime kit and [32P]dCTP (Du Pont-New England Nuclear, 800 Ci/mmol).

RESULTS

Expression of Cyp1a mRNA in Cultured Rat Arterial SMC—Total RNA from confluent Wistar-Kyoto pup and adult SMC cultures was blotted and probed with the rat Cyp1a probe, pc1. Fig. 1A shows the resulting autoradiogram. Pup SMC express high levels of Cyp1a mRNA compared with adult SMC. Densitometric analysis of the autoradiogram indicated at least a 10-fold difference in the steady-state abundance of this mRNA in pup versus adult SMC. A similar relative difference in mRNA levels was observed between cultured rat carotid intimal and medial SMC. Intimal SMC were isolated from rat carotid artery 2 weeks following balloon catheter injury (5). Medial SMC were derived from the uninjured, contralateral carotid of the same rat. The same results were obtained when RNAs from a second pair of cultured intimal and medial SMC were probed with the Cyp1a probe (data not shown). The size of the major hybridizing mRNA was 2.7 kb in all cell types and is the expected size for a bonafide Cyp1a mRNA transcript (23). Rehybridization of this blot with a probe for an invariant “housekeeping” gene, glyceraldehyde-3-phosphate dehydrogenase, indicated that equivalent amounts of mRNA were applied to each lane (data not shown, see also Fig. 3).

To determine whether the difference in developmental expression of Cyp1a mRNA observed in pup and adult SMC was a general phenomenon, or unique to Wistar-Kyoto rats, total RNA was also prepared from confluent cultures of pup or adult SMC derived from spontaneously hypertensive (SHR) and Sprague-Dawley (SD) rats. Fig. 1B demonstrates that Cyp1a mRNA levels are consistently higher in pup SMC compared with adult cells independent of the strain of rat used. Interestingly, absolute levels of mRNA varied among strains. This is consistent with the observation that Cyp1a expression is highly species and strain specific (27).

To determine whether the differential expression of Cyp1a mRNA in cultured SMC.

![Fig. 1. Cyp1a mRNA in cultured SMC.](image-url)

A. pc1 probe indicates the position of migration of the Cyp1a mRNA at 2.7 kilobases.
in pup versus adult cells was dependent on the growth state of the cell, the following experiment was performed. Pup or adult cells were grown to confluence and growth-arrested by treating for 72 h with either low serum (0.5% calf serum) or serum depleted of mitogens (5% C-Sephadex-absorbed plasma-derived serum). Cells were then treated with either 20% calf serum or 5 ng/ml platelet-derived growth factor as described in the figure legend. As shown in Fig. 2, confluent, growth-arrested cells as well as cells treated for 8 h with a mitogenic stimulus showed little change in CypIal mRNA level.

Cycloheximide Dramatically Elevates CypIal mRNA in Adult SMC—To determine whether active protein synthesis was required for the difference observed in CypIal expression levels, pup and adult cells were treated with cycloheximide prior to RNA isolation. Fig. 3 illustrates the results of this treatment. Pup SMC showed little change in CypIal mRNA levels in response to cycloheximide treatment whereas adult SMC displayed a dramatic increase in the abundance of the CypIal mRNA under the same conditions (panel A). Densitometry of the 2.7-kb CypIal band indicated at least a 10-fold increase in the steady-state level of this transcript in adult SMC after exposure to 10 μg/ml cycloheximide, thus reaching a level comparable to that seen in untreated pup cells. Less than a 2-fold increase in CypIal mRNA was seen in cycloheximide-treated pup cells. A high molecular weight band at approximately 4 kb, most likely representing an unspliced nuclear precursor of CypIal mRNA (28), was also induced in adult SMC by cycloheximide. That cycloheximide was effective in pup cells was demonstrated by reprobing the filter with a cDNA probe for c-myc, an mRNA known to be cycloheximide-inducible in both pup and adult SMC (3). The autoradiogram shown in panel B indicates that cycloheximide was similarly effective in elevating c-myc mRNA levels in both pup and adult cells. Furthermore, treatment of the cells with a second protein synthesis inhibitor, puromycin, gave identical results (data not shown). The changes in CypIal mRNA levels were specific since the abundance of mRNA for glyceraldehyde-3-phosphate dehydrogenase (panel C) did not change with cycloheximide treatment.

Transcription of the CypIal Gene in Pup and Adult SMC—A difference in the steady-state levels of CypIal mRNA in pup and adult cells could be due to (a) enhanced transcription of the CypIal gene in pup cells, (b) decreased stability of the CypIal mRNA in adult cells, or (c) a combination of both. A transcriptional mechanism of action was suggested by the effect of actinomycin D on cycloheximide induction of CypIal mRNA in adult SMC. As shown in Fig. 4, cotreatment of adult cells with actinomycin D and cycloheximide prevented the observed increases in CypIal mRNA caused by cycloheximide alone (Fig. 4B, left panel) and had little effect over actinomycin D alone on the level of CypIal mRNA in pup cells (Fig. 4B, right panel). The autoradiograms in Fig. 4A and B, left panels were purposely overexposed in order to clearly illustrate the effect of the treatments on the low levels of CypIal mRNA in adult cells.

These data strongly suggested that the elevated steady-state level of CypIal mRNA in cycloheximide-treated adult SMC were due to an enhanced transcription rate of the CypIal gene in pup and adult SMC. Cells were treated with 0, 10, or 20 μg/ml cycloheximide (CHX) for 3 days to arrest growth. The cells were then stimulated with either 20% fetal calf serum (20% CS) or 5% CM-Sephadex-absorbed, plasma-derived serum (5% CMS-PDS) for 3 days to arrest growth. The cells were then stimulated with either 20% fetal calf serum (20% CS) or 5% CM-Sephadex-absorbed, plasma-derived serum (5 ng/ml PDGF) for 8 h. 10 μg of total RNA derived from cells after each treatment were Northern blotted and probed with [32P]pC1. PDGF, platelet-derived growth factor.

Fig. 2. Effect of SMC growth state on CypIal mRNA levels. Confluent cultures of adult (A) or pup (B) SMC were treated with 0.5% calf serum (0.5% CS) or 5% CM-Sephadex-absorbed, plasma-derived serum (5% CMS-PDS) for 3 days to arrest growth. The cells were then stimulated with either 20% fetal calf serum (20% CS) or 5 ng/ml platelet-derived growth factor plus 5% Sephadex-absorbed, plasma-derived serum (5 ng/ml PDGF) for 8 h. 10 μg of total RNA derived from cells after each treatment were Northern blotted and probed with [32P]pC1. PDGF, platelet-derived growth factor.

Fig. 3. Effect of cycloheximide on CypIal mRNA levels in pup and adult SMC. Cells were treated with 0, 10, or 20 μg/ml cycloheximide (CHX) for 3 days followed by total RNA isolation. Twenty μg of total RNA from each sample were Northern blotted and probed with either [32P]pC1 to detect CypIal mRNA (upper panel), [32P]c-myc probe (middle panel) or [32P]glyceraldehyde-3-phosphate dehydrogenase probe (lower panel).

Fig. 4. Stability of CypIal mRNA in pup and adult SMC. A, pup and adult SMC were treated with 40 μg/ml actinomycin D (actinomycin). Total RNA was isolated after 0, 2, and 4 h of treatment. 10 μg/ml RNA were Northern blotted and probed with [32P]pC1. B, pup and adult SMC were treated simultaneously with 40 μg/ml actinomycin plus 10 μg/ml cycloheximide (ACT+CHX). RNA was isolated after 0, 2, and 4 h of treatment. 10 μg/ml RNA was Northern blotted and probed with [32P]pC1. Exposures times were 16 h for pup RNAs and 48 h for adult RNAs.
CypIal gene in these cells compared with untreated cells. To definitively prove this, as well as address the mechanism responsible for the difference in the basal expression of CypIal in pup versus adult SMC, nuclear transcription assays were performed. Pup and adult SMC were treated with 20 μg/ml cycloheximide or vehicle alone for 2 h, and nuclei were isolated. Total RNA was also prepared from identically treated plates, Northern blotted, and probed for CypIal or fibronectin mRNA using the pC1 or rif-1 cDNAs, respectively (Fig. 5A). The autoradiogram indicates that, at the time of nuclear isolation, pup and adult SMC displayed the expected difference in CypIal mRNA levels, and that the 2 h cycloheximide treatment was effective in elevating CypIal mRNA in adult cells (left panel). Treatment of pup cells with cycloheximide showed approximately a 2-fold increase in CypIal mRNA level (not shown). The abundance of fibronectin transcripts showed little variation in the SMC samples regardless of source or treatment (right panel). Radiolabeled nascent RNAs from nuclear transcription assays were then hybridized to the various plasmids shown in Fig. 4B. Very little CypIal gene transcription was detected using this assay when the source of nuclei was adult SMC (middle panel). Radiometric quantitation of the mRNA bound to the pC1 slot indicated an undetectable level of transcription in adult nuclei (<5 cpm bound after background subtraction, see “Materials and Methods” for calculations). In contrast, significant transcription of the CypIal gene was detected in nuclei derived from pup SMC (left panel) and adult SMC treated with cycloheximide (right panel). Transcriptional rates of 101 ± 44 ppm and 166 ± 26 ppm were calculated for pup and cycloheximide-treated adult SMC, respectively. Very little change in the transcription rate was detected in cycloheximide-treated pup nuclei compared with untreated pup nuclei (data not shown). As expected, transcription of the fibronectin gene remained unchanged in response to cycloheximide.

**DISCUSSION**

Expression of the CypIal gene in cultured arterial SMC depends on the age of the donor animal. Pup SMC transcribe the CypIal gene at a higher rate than adult SMC resulting in elevated levels of CypIal mRNA in pup SMC. Treatment of adult SMC with cycloheximide increased CypIal gene transcription in adult cells with only minimal effect in pup SMC. These data are consistent with the presence of a labile repressor of CypIal gene transcription in adult SMC which is lacking, inactive, or at substantially reduced level in pup SMC. Although the presence of a positive regulatory element in pup SMC cannot be ruled out by these studies, treatment of adult cells with pup conditioned media provided no evidence for an autocrine, diffusible element that maintains increased basal levels of CypIal mRNA expression in pup SMC.

Evidence for a labile repressor of CypIal gene transcription has been described in mouse (30, 31) as well as human (32) hepatoma cell lines. In these systems, cycloheximide increases transcription of the CypIal gene but only in the presence of a transcriptional inducing agent, dioxin. Dioxin exerts its transcriptional effect by binding a cytosolic dioxin receptor molecule, translocating into the nucleus, and interacting with a specific nucleotide enhancer sequence designated a xenobiotic responsive element (33, 34), thereby activating gene transcription. Interestingly, the cycloheximide effect in hepatoma cells requires functional cytosolic dioxin receptors since mutant hepatoma cells defective in these receptors did not respond to cycloheximide (31). However, cycloheximide induction in adult SMC does not appear to depend on the

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2 C. M. Giachelli, M. W. Majesky, and S. M. Schwartz, unpublished observation.
presence of an additional exogenous-inducing agent. Regardless of the mechanism, the different effects of these treatments in vascular SMC compared with hepatoma cells indicates substantial cell-type specific regulation of the CypIal gene.

That both pup and adult SMC respond to a dioxin-like inducer of CYP1A1 gene transcription, β-napthoflavone, suggests that both contain a functional cytosolic dioxin receptor. However, the time course for induction of the CypIal in either pup or adult SMC is strikingly different from that reported for other cell lines. Cresteil et al. (35), measured CypIal mRNA accumulation in response to 2,3,7,8-tetrachlorodibenzo-p-dioxin in a breast carcinoma (MCF-7) and liver tumor (HepG2) cell lines. In both cases, maximum CypIal levels were observed only after approximately 24 h of treatment. Jaiswal et al. (36) confirmed these findings in MCF-7 cells, as well as showing that benzo(a)anthracene elicited the same effect. In contrast, both pup and adult cells showed maximal CypIal mRNA expression after only 2 h of treatment with β-napthoflavone. These data, together with the observation that a novel β-napthoflavone-responsive element exists in the glutathione S-transferase Ya subunit gene (37), caution us to consider such a possibility in rat SMC.

Represors of gene transcription have been described for a number of other systems including the interferons (38), metallothioneins (39), and heat shock genes (40). Adenovirus E1A products also appear to contain repressor-like activities which have been shown to inhibit transcription from muscle-specific promoters (41). Transcriptional repression of the smooth muscle α-actin gene accompanies striated muscle cell maturation (42) and may be particularly relevant to the present observations if we assume that smooth muscle, like striated muscle, undergoes a specific program of genetic determination during development (1). Control of tissue or stage-specific gene transcription by activation (or conversely down-regulation) of a repressor molecule may be a common mechanism for control of cell development.

Recent work from this laboratory has suggested a novel link between differentiation and smooth muscle cell proliferation. We have observed a number of properties that are stable in culture and shared with cultured SMC derived either from pup rats or from the neointima formed after balloon injury. These properties, absent from the usual SMC cultured from the adult aorta, include the ability to grow in medium prepared without platelet release, secretion of mitogenically active platelet-derived growth factor-like molecules, and synthesis of platelet-derived growth factor-like mRNA (2-5). In addition, the pronounced epithelioid, monolayer pattern of cell growth of pup or intimal SMC is easily distinguished from the spindle-shaped "hill and valley" growth pattern of adult SMC (2-4). All of these differences are stable in culture, implying that some sort of heritable, genetic difference exists between SMC in the rat pup aorta and the adult aorta and that this same difference is somehow remanifest when the SMC population of the aorta is stimulated to form a neointima. Our hypothesis is that a change in SMC differentiation occurs during the first few weeks after birth and that a residual, undifferentiated population contributes to neointimal proliferation following balloon injury. The differential pattern of expression reported here for the CypIal gene supports this hypothesis. Furthermore, these studies clarify, at least for the CypIal gene, the molecular basis for the observed pup versus adult CypIal phenotype.

What role might CypIa1 play in early SMC development or in cells induced to proliferate in response to injury? A role for P-450-dependent metabolism in the pathogenesis of atherosclerosis has been implicated by a number of reports showing that typical P-450-inducing agents and substrates, such as 3-methylcholanthrene, can increase atherosclerotic lesion size in a variety of test species (43-46). Significantly, Faiglen and co-workers (47) have employed mouse strains with (Ah+/+) and without (Ah/-) an intact dioxin receptor to show a correlation between the pleiotypic response to 3-methylcholanthrene-type inducing agents and susceptibility to 3-methylcholanthrene-induced atherosclerosis. Studies in pigeons also indicate a positive correlation between high P-450 inducibility and a tendency toward atherosclerosis (48). The fact that intimal SMC express a pup-like phenotype with regard to CypIal mRNA expression lends support to the idea that the cytochrome P-450 system may be involved in the pathogenesis of cardiovascular disease. We are currently performing experiments to determine the expression of CypIal in vivo in order to address this question.

Last, it should not be ruled out that CypIal may have, in addition, a novel function in SMC. Evidence that CypIal may be important in cellular differentiation includes its very early expression in the 7-day-old embryonic mouse, increased expression in the regenerating rat liver after partial hepatectomy, and enhanced expression in F9 carcinoma cells induced to differentiate in response to retinoic acid (49). High constitutive expression of CypIal has also been reported in several human tumor cell lines (50). Alternatively, CypIal expression in SMC may be part of a pleiotypic response to other critical, as yet unknown, event in SMC development, perhaps involving some endogenous role for the dioxin receptor (programmed cell death?, cell growth and proliferation?). Which, if any of these possibilities is important in cardiovascular processes awaits further analysis of CypIa1 in the normal and diseased vasculature.

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