Disruption of endogenous regulator homeostasis underlies the mechanism of rat CYP1A1 mRNA induction by metyrapone

Joanna L. HARVEY¹, Alan J. PAINE and Matthew C. WRIGHT²

Department of Toxicology, St. Bartholomew's and the Royal London School of Medicine and Dentistry, Charterhouse Square, London EC1M 6BQ, U.K.

The transcriptional induction of the cytochrome *P*-450 1A1 (CYP1A1) gene by xenobiotics such as polyaromatic hydrocarbons is dependent on their interaction with the aryl hydrocarbon receptor. Administration of the structurally unrelated compounds metyrapone (a cytochrome *P*-450 inhibitor) or dexamethasone (a glucocorticoid) to male rats does not induce hepatic CYP1A1 mRNA. However, administration of both metyrapone and dexamethasone to male rats results in the induction of hepatic CYP1A1 mRNA expression. The induction response is mimicked *in vitro* in cultured rat hepatocytes by the addition of metyrapone and dexamethasone to a serum-free culture medium, suggesting that these compounds act directly on the liver *in vivo* to effect hepatic CYP1A1 mRNA induction. An examination of the characteristics of CYP1A1 induction by metyrapone and dexamethasone in combination *in vitro* indicate that at least 6 h of treatment is required for detectable levels of CYP1A1 mRNA to accumulate in hepatocytes. In contrast, β -naphthoflavone, which is known to bind to the aryl hydrocarbon receptor to effect CYP1A1 gene expression, induces detectable levels of CYP1A1 mRNA within 2 h of treatment. CYP1A1 mRNA is also induced when hepatocytes are treated with metyrapone in combination with the protein synthesis inhibitor cycloheximide but not with dexamethasone in combination with cycloheximide, indicating that CYP1A1 mRNA induction is strictly dependent on the presence of metyrapone and suggesting that the metyrapone-associated induction of CYP1A1 mRNA is dependent on a loss of a constitutively expressed protein that functions to suppress CYP1A1 gene expression. The role of dexamethasone in metyrapone-associated induction of CYP1A1 is probably mediated through the gluco-

corticoid receptor since the glucocorticoid receptor antagonist RU486 reduces the levels of CYP1A1 mRNA induced by metyrapone and dexamethasone in combination. Increasing the levels of the photosensitizer riboflavin present in the culture medium 10-fold and exposure to light increases the levels of CYP1A1 mRNA induced by metyrapone and dexamethasone in combination *in vitro*, suggesting that photoactivation of inducing medium constituent(s) might be required for induction. Failure to induce CYP1A1 mRNA by co-administration of metyrapone and dexamethasone in hepatocytes cultured in a balanced salt solution with or without photoactivation indicates that induction is dependent on a photoactivated component of the culture medium and not on metyrapone or dexamethasone alone. The addition of tryptophan in the presence of riboflavin to the balanced salt solution restores CYP1A1 mRNA induction by metyrapone alone and induction is increased when medium is exposed to light, indicating that induction is dependent on tryptophan photoactivation *in vitro*. Metyrapone failed to compete with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin for specific binding to the aryl hydrocarbon receptor in rat liver cytosolic fractions. These results suggest that CYP1A1 might be induced in rats by metyrapone through an indirect mechanism associated with an elevation in the level of an endogenously generated inducer such as photoactivated product(s) of tryptophan and not because of metyrapone's interacting with the aryl hydrocarbon receptor. The dependence of CYP1A1 induction on dexamethasone or cycloheximide suggests that derepression by a glucocorticoid receptor-modulated negative-acting factor of CYP1A1 gene expression might be critical to induction by metyrapone.

INTRODUCTION

The term cytochromes *P*-450 (CYP) describes a gene superfamily of haemoproteins that mediate the metabolism of many xenobiotics [1]. The CYP1A1 gene product is prominent in the metabolism and activation of carcinogenic polycyclic aromatic hydrocarbons (PAHs), many of which also induce the expression of this gene [2]. Many tissues express constitutively low levels of CYP1A1 and expression is induced through the interaction of

inducers with the aryl hydrocarbon receptor (AhR), a basic helix-loop-helix protein component of a transcription factor complex present in the cytosolic fraction of many tissues when unliganded or inactivated [3–5]. The polychlorinated dioxin 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is a potent inducer of CYP1A1 and binds with relatively high affinity to the AhR compared with other PAH-binding proteins [6]. Accordingly it is a model compound for examining the functional role of the AhR in cellular processes. It has been established that TCDD's binding

Abbreviations used: CYP, cytochrome *P*-450 (the nomenclature employed for cytochrome *P*-450 is that recommended [1]); MET, metyrapone [2-methyl-1,2-bis(3-pyridyl)-1-propanone]; DEX, dexamethasone (9-fluoro-11 β ,17,21-trihydroxy-16 α -methylpregna-1,4-diene-3,20-dione); β -NF, β -naphthoflavone (5,6-benzoflavone); α -NF, α -naphthoflavone (7,8-benzoflavone); RU486, mepipristone [17 β -hydroxy-11 β -(4-dimethylaminophenyl)-17 α -1-propynyl-oestra-4,9-dien-3-one]; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; GR, glucocorticoid receptor; AhR, aryl hydrocarbon receptor; PAH, polyaromatic hydrocarbon; GRE, glucocorticoid response element; [LR]_e^{max}, maximum derived ligand-receptor concentration determined experimentally, equivalent to receptor concentration assuming 1:1 stoichiometric interaction; HBSS, Hanks balanced salt solution; BP, benzo[*a*]pyrene.

¹ Present address: Department of Biochemistry and Molecular Biology, Royal Free Hospital School of Medicine, Rowland Hill St, London NW3 2PF, U.K.

² To whom correspondence should be sent. Present address: University Medicine, Level D, South Block, Southampton General Hospital, Tremona Rd, Southampton SO16 6YD, U.K.

to the AhR results in the dissociation of the complex of proteins associated with the AhR, such as heat shock protein 90 [7] and others [8]. Association of the AhR with another basic helix-loop-helix protein, the aryl hydrocarbon nuclear translocator, facilitates nuclear localization and is required for association with specific DNA sequences known as dioxin response elements or xenobiotic response elements [9,10]. The presence of xenobiotic response elements upstream of the CYP1A1 gene in mouse, rat and human has been demonstrated to increase the transcription of the CYP1A1 gene [11–13]. The pivotal role of the AhR in the mechanism of CYP1A1-inducible expression in the mouse is confirmed by a loss in TCDD-inducible expression in ‘knock-out’ AhR^{-/-} mice containing a disrupted AhR gene [14]. However, there remains a dispute about the role of other PAH-binding proteins in the CYP1A1 induction response and suggestions that there might be an AhR-independent mechanism of CYP1A1 gene induction [15]. The CYP1A1 inducer benzo[a]pyrene (BP) has been reported by some groups to bind only to the 4 S PAH-binding protein in rat cytosol, although the identity of this protein still remains to be confirmed [16–19].

Previous work in this laboratory has shown that metyrapone (MET) is a ‘phenobarbitone-type’ inducer in that it induces the CYP2B and CYP3A subfamilies, but not the CYP1A subfamily, in rats [20,21]. The ability of MET to maintain the spectrophotometrically detectable levels of CYP in cultured hepatocytes [22] is associated with a transcriptional induction of CYP3A1/3A23 [23] and a stabilizing effect on existing CYP protein through liganding the haem prosthetic group [22,24,25]. CYP2B is induced to only low levels by MET *in vitro* in hepatocytes cultured in serum-free medium (O. Boocock, A. J. Paine and M. C. Wright, unpublished work), probably due to the absence of an extracellular matrix [26] from the culture system employed in these studies. However, MET also induces CYP1A protein [20] and CYP1A1 mRNA levels [27] (but not CYP1A2 mRNA levels [28]) in hepatocytes (cultured in serum-containing medium), in apparent contrast with observations *in vivo*. Several compounds have been demonstrated to be CYP1A1 inducers, including carbaryl [29], brevitoxin-6 [30], myristicin [31] and omeprazole [32], although they are either unexpected ligands for the AhR or show no detectable affinity for the AhR. The induction of CYP1A1 mRNA by MET is therefore examined under fully defined conditions because this compound and its known metabolites do not have the characteristic structure of classical AhR ligands [33]. The interaction of the glucocorticoid dexamethasone (DEX) is examined because glucocorticoids are common additions to hepatocyte culture media and might explain the difference between the effects of MET observed *in vitro* and *in vivo* with respect to CYP1A1 gene expression.

MATERIALS AND METHODS

Animals and materials

Male Sprague–Dawley CD rats purchased from Charles River Ltd (Margate, Kent, U.K.) at a body weight of 250–300 g were used in all studies. Animals were housed for at least 1 week before use in an air-conditioned environment of 20 ± 3 °C and a humidity of 50 ± 10 % with a 12 h light/12 h dark cycle. Food (rat maintenance diet no. 1; SDS Witham, Essex, U.K.) and water were available *ad libitum*.

17 β -Hydroxy-11 β -(4-methylaminophenyl)-17 α -1-propynyl-oestra-4,9-dien-3-one (RU486) and TCDD were generously supplied by Roussel-Uclaf (Romainville, France) and Dr. A. Smith (MRC Toxicology Unit, Leicester, U.K.). MET and DEX were purchased from the Sigma Chemical Co. The rat CYP1A1 and rat actin cDNAs were provided by John Fagan (Maharishi

International University, Fairfield, IA, U.S.A.) [34] and Dr. G. Allenby (Hoffman La Roche, Nutley, NJ, U.S.A.). Radio-labelled [1,6-³H]TCDD (24.4 Ci/mmol) was purchased from ChemSyn Labs (Lenexa, KS 66215, U.S.A.) [G-³H]benzo[a]pyrene (63 Ci/mmol) and [α -³²P]dCTP (3000 Ci/mmol) were purchased from Amersham (Little Chalfont, Bucks., U.K.).

Animal dosing

Substances were administered to rats by intraperitoneal injection (250 mg of MET/kg body weight and/or 5 mg of dexamethasone-21-phosphate/kg body weight). Each compound was dissolved in saline vehicle. Control animals received saline vehicle only. Treatment was repeated 24 h after the first dose and the animals were killed by CO₂ asphyxiation 6 h after the second and final dose for analysis of mRNA expression.

Hepatocyte isolation and culture

Single-step collagenase live perfusion was performed as described [35]. In brief, each rat was anaesthetized by intraperitoneal injection with 60 mg sodium pentobarbitone (Rhône Poulenc, Dagenham, Essex, U.K.) per kg of body weight and a heparin-filled 16G cannula was inserted into the portal vein. After the cannula had been secured by ligation, a cut was made in the posterior vena cava and the liver was perfused with Ca²⁺/Mg²⁺-free Hank's balanced salt solution (HBSS) [0.14 M NaCl/5.4 mM KCl/0.34 mM Na₂HPO₄·7H₂O/0.44 mM KH₂PO₄/5.6 mM D-glucose/15.7 mM NaHCO₃]. The perfusate was gassed with O₂/CO₂ (19:1) before being passed through the liver at a pH of 7.4 and a temperature of 37 °C. After the liver had been cleared of blood and excised from the body cavity, it was perfused with HBSS containing 0.025 % collagenase H (Boehringer Mannheim, Lewes, East Sussex, U.K.) and recirculation continued for 30 min. The cells were dispersed in HBSS containing 2 % (w/v) BSA, filtered through 120 μ m nylon mesh and centrifuged at 50 g for 3 min. The cells were resuspended in ‘modified WME’ culture medium [William's Medium E (Flow Laboratories, Irvine, Scotland, U.K.) supplemented with 2 nM insulin, 10 nM glucagon and 50 μ g/ml gentamycin] and washed twice further in modified WME by centrifugation at 50 g for 3 min. Viable hepatocytes (1.8 × 10⁷) were suspended in 20 ml of modified WME further supplemented with 1 μ g/ml fibronectin to promote substratum attachment, and seeded on 150 mm diameter collagen-coated (130 μ g per plate) plastic plates in a humidified air/CO₂ (19:1) atmosphere at a temperature of 37 °C.

For analysis of the effect of cycloheximide on CYP1A1 mRNA expression, hepatocytes were seeded in modified WME as outlined above. After 4 h of culture, the medium was replaced with medium containing cycloheximide and/or any additional treatments. For examination of the effect of inducers on hepatocytes cultured in a balanced salt solution, hepatocytes were also seeded in modified WME. After 4 h, culture medium was removed and attached hepatocytes were washed extensively with HBSS containing 1.3 mM CaCl₂ and 0.81 mM MgSO₄ (HBSS+Ca/Mg) and then incubated with HBSS+Ca/Mg supplemented with required treatments. Culture medium illumination was achieved essentially as described previously [36,37]. In brief, medium in glass bottles was exposed to light illumination from a 60 W tungsten lamp at a distance of 17 cm for 30 min before use.

Isolation of RNA and Northern blotting

RNA was isolated from whole liver and cultured hepatocytes by using RNazol (Biogenesis, Bournemouth, Hants., U.K.) essentially as outlined in the manufacturer's instructions. Briefly,

RNA was extracted from RNAzol homogenates with chloroform, precipitated with propan-2-ol and washed with 75% (v/v) ethanol. The RNA was resuspended in water at approx. 4–8 mg/ml and aliquots were stored at -20°C . RNA was electrophoresed on 1.5% (w/v) agarose gel in 20 mM Mops buffer, pH 7.0, containing 5 mM sodium acetate, 1 mM EDTA and 1.9% (w/v) formaldehyde. Integrity and equal loading of RNA were ascertained by observing ethidium bromide-stained 28 S and 18 S rRNA bands under UV illumination. The RNA was transferred overnight from gels to nylon membranes (Hybond-N, Amersham) by capillary action and then cross-linked to the membrane by UV irradiation (306 nm, 120 mJ). Hybridizations with cDNA probes were performed with QuikHyb (Stratagene) in accordance with the manufacturer's instructions. Membranes were washed twice at room temperature with $2 \times \text{SSC}/0.1\%$ SDS for 30 min [$20 \times \text{SSC}$ in 3 M NaCl/0.3 M sodium citrate (pH 7.0)] followed by a wash in $0.1 \times \text{SSC}/0.1\%$ SDS for 30 min. A high-stringency wash (5–10 min) was performed between 40 and 55°C in $0.1 \times \text{SSC}/0.1\%$ SDS before membranes were wrapped in Saran wrap and subjected to autoradiography at -70°C .

Receptor–ligand binding studies

Hepatic cytosolic fractions were prepared after the removal of blood from the liver by perfusion with ice-cooled HBSS essentially as outlined for collagenase perfusion above. The liver was disrupted in HEDG buffer [23 mM Hepes buffer (pH 7.6)/1 mM EDTA/10% (v/v) glycerol/1 mM dithiothreitol] with a Potter–Elvehjem homogenizer; the resultant homogenate was centrifuged at $14000g$ for 20 min at 2°C . The supernatant was centrifuged at $100000g$ for 2 h at 2°C and the supernatant (cytosol) was frozen in aliquots at -70°C . The total cytosolic protein concentration was determined by the method of Lowry et al. [38].

Cytosolic fractions were defrosted as needed and diluted to 10 mg/ml protein with HEDG buffer. Aliquots were incubated on ice with radioactive ligand with or without excess unlabelled ligand or potential competitors until specific radiolabelled binding had reached equilibrium, as determined in separate studies. Specifically and non-specifically labelled cytosolic protein was separated from free radioligand by gel filtration with Sephadex G25 PD-10 in disposable columns (Pharmacia). The radioactivity was determined by liquid scintillation. The levels of specifically radiolabelled protein were determined by comparing radiolabelled protein radioactivity with samples incubated with radiolabelled ligand plus a 200-fold molar excess of unlabelled ligand. For the determination of the ligand binding parameters K_d and $[\text{LR}]_e^{\text{max}}$ (receptor concentration), a range of radiolabelled ligand concentrations were used and binding data were fitted by least-squares nonlinear regression with the ENZFITTER program [39]. An aliquot of the incubation was removed before separation of free ligand from protein and the radioactivity was determined in order to determine accurately the concentration of labelled ligand.

Owing to the high levels of non-specific binding of TCDD radiolabel to cytosolic protein, specific and non-specifically radiolabelled TCDD cytosolic protein incubated with or without excess unlabelled TCDD or metyrapone was subjected to gel filtration (after removal of free ligand with Sephadex G25 PD-10 columns) on a Superose 12HR 10/30 FPLC column (Pharmacia) with HEDG as the mobile phase. The eluate was monitored for protein at 280 nm and fractions were collected for analysis of radioactivity by liquid scintillation.

RESULTS

Induction of hepatic CYP1A1 mRNA in rats and hepatocyte cultures treated with MET and DEX

Figure 1 indicates that the administration of both MET and DEX (MET/DEX) to adult male rats resulted in the induction, from undetectable levels, of hepatic CYP1A1 mRNA. In contrast, the administration to rats of either MET or DEX alone did not induce CYP1A1 mRNA (Figure 1). Hepatocytes cultured in a serum-free medium with MET/DEX also induced CYP1A1 mRNA but not in the presence of either compound alone (Figure 2). Figure 2 shows that the AhR ligand and CYP1A1 inducer β -naphthoflavone (β -NF) [40] alone induced CYP1A1 mRNA in this culture system and that DEX increased the levels of CYP1A1 mRNA induced by β -NF. The enhancing effects of DEX on β -NF induction of CYP1A1 mRNA contrasts with induction by MET/DEX in that both compounds must be present in the latter situation. An examination of the time course for the induction of CYP1A1 mRNA by either MET/DEX or β -NF indicates that β -NF induced detectable levels of mRNA within 2 h of treatment (Figure 3a), whereas MET/DEX treatment required between 6 and 24 h of treatment (Figure 3b).

It should be noted that the levels of CYP1A1 mRNA induced by MET/DEX *in vitro* (when compared with the levels of CYP1A1 mRNA induced by β -NF in the same experiment) were variable between separate experiments (compare responses in Figures 5 and 6). However, the concentration-dependent effect(s) of DEX on the induction of CYP1A1 mRNA with MET remained similar between experiments in that 50% maximal induction was observed with DEX in the nanomolar range, as observed for DEX enhancement of β -NF-dependent CYP1A1 mRNA induction (results not shown).

Effect of cycloheximide on the induction of CYP1A1 mRNA by MET/DEX in cultured hepatocytes

Figure 4 indicates that addition of the protein synthesis inhibitor cycloheximide [41] to culture medium did not induce CYP1A1

Figure 1 Effect of MET and DEX administration to rats on the expression of hepatic CYP1A1 mRNA

Northern blot of total RNA (20 μg per lane) from liver after the administration of MET and DEX to rats, as outlined in the Materials and methods section, and hybridized to CYP1A1 cDNA probe. Lane 1, saline vehicle control; lane 2, 250 mg/kg MET; lane 3, 5 mg/kg DEX-21-phosphate; lane 4, 250 mg/kg MET plus 5 mg/kg DEX-21-phosphate. Equal loading was confirmed by reference to the ethidium bromide staining of RNA after transfer to nylon membrane. Results are typical of three separate studies.

Figure 2 Effect of MET, DEX and β -NF on the expression of CYP1A1 mRNA in hepatocytes cultured in modified WME

Northern blot of total RNA (20 μ g per lane) from hepatocytes cultured for 24 h in modified WME and additions as noted. Lane 1, isolated hepatocytes before treatment; lane 2, solvent only; lane 3, 20 μ M β -NF; lane 4, 500 μ M MET; lane 5, 200 nM DEX; lane 6, 500 μ M MET plus 200 nM DEX; lane 7, 20 μ M β -NF plus 200 nM DEX. The gel in the upper panel was probed with the CYP1A1 cDNA; that in the lower panel was probed with the actin cDNA (note that the low levels of actin mRNA in isolated cells were due to a temperature-dependent induction of an RNase directed at specific mRNA species that is induced in hepatocytes when they are isolated [67], and were not due to less RNA in isolated cells, as determined by ethidium bromide staining of rRNA). Results are typical of at least ten separate experiments.

mRNA levels in hepatocytes but that when it was combined with MET there was induction of CYP1A1 mRNA. The results presented in Figure 4 also indicate that the co-incubation of cycloheximide with DEX did not induce CYP1A1 mRNA. Comparison of the levels of CYP1A1 mRNA induced by MET/DEX and MET/DEX in the presence of cycloheximide suggests that there was no simple additive effect of DEX and cycloheximide on the apparent MET-dependent induction of CYP1A1 mRNA (Figure 4).

Effect of the RU486 and α -naphthoflavone (α -NF) on the induction of CYP1A1 mRNA by β -NF and MET/DEX in cultured hepatocytes

The results presented in Figure 5 indicate that the glucocorticoid and progesterone receptor antagonist RU486 [42] did not induce CYP1A1 mRNA expression in cultured hepatocytes. However, when RU486 was added to MET-treated hepatocytes, CYP1A1 mRNA was induced although the level of induction was low compared with MET/DEX-treated hepatocytes (Figure 5). The addition of RU486 to MET/DEX-treated hepatocytes decreased the levels of CYP1A1 mRNA induced by MET/DEX alone, whereas RU486 has no effect on the levels on CYP1A1 mRNA induced by β -NF (Figure 5).

α -NF has been reported to be an AhR antagonist [43] or a weak AhR agonist [44]. In our hands, α -NF alone induced the levels of CYP1A1 mRNA to levels comparable to those achieved by β -NF, and they were enhanced by co-incubation with DEX (Figure 5). Interestingly, there is an increased level of CYP1A1 mRNA induction when cultured hepatocytes were treated with

Figure 3 Time course for the induction of CYP1A1 mRNA by β -NF (a) and DEX/MET (b) in hepatocytes cultured in modified WME

Northern blot of total RNA (20 μ g per lane) from hepatocytes cultured for the times indicated in modified WME and additions as noted. (a) Lane 1, isolated hepatocytes before treatment; lanes 2, 4, 6 and 8, solvent only; lanes 3, 5, 7 and 9, 20 μ M β -NF. The gel in the upper panel was probed with the CYP1A1 cDNA; that in the lower panel was probed with the actin cDNA. (b) Lane 1, isolated hepatocytes before treatment; lanes 2, 6, 10 and 14, solvent only; lanes 3, 7, 11 and 15, 500 μ M MET; lanes 4, 8, 12 and 16, 200 nM DEX; lanes 5, 9, 13 and 17, 500 μ M MET plus 200 nM DEX. The gel in the upper panel was probed with the CYP1A1 cDNA; that in the lower panel was probed with the actin cDNA. Results are typical of at least three separate experiments.

both α -NF and MET/DEX compared with the levels induced by each treatment alone. In contrast, no additive effect was observed with α -NF and β -NF (Figure 5).

Effect of riboflavin, light illumination and the presence of histidine or tryptophan on the induction of CYP1A1 mRNA by DEX/MET

The results presented in Figure 6 indicate that the induction of CYP1A1 mRNA was increased when hepatocytes were treated with MET/DEX in medium subjected to photoactivation (by increasing the concentration of the photosensitizer riboflavin present in WME from 0.27 to 2.7 μ M and by exposure to light) compared with medium (supplemented with additional riboflavin) that had received minimal light exposure. In addition, under these conditions, MET alone consistently induced CYP1A1 mRNA when present in medium supplemented with additional riboflavin and exposed to light (Figure 6).

The results presented in Figure 7 indicate that MET or MET/DEX did not induce CYP1A1 mRNA when hepatocytes

Figure 4 Effect of cycloheximide on the induction of CYP1A1 mRNA in hepatocytes cultured in modified WME

Northern blot of total RNA (20 μ g per lane) from hepatocytes cultured for 4 h in modified WME to allow attachment followed by medium renewal and treatment for a further 20 h with additions as noted. Lane 1, isolated hepatocytes before treatment; lane 2, hepatocytes cultured for 4 h without additions to culture medium; lane 3, solvent only; lane 4, 500 μ M MET; lane 5, 200 nM DEX; lane 6, 500 μ M MET plus 200 nM DEX; lane 7, 20 μ M β -NF; lane 8, 8 μ M cycloheximide; lane 9, 8 μ M cycloheximide plus 500 μ M MET; lane 10, 8 μ M cycloheximide plus 200 nM DEX; lane 11, 8 μ M cycloheximide plus 500 μ M MET plus 200 nM DEX. The gel in the upper panel was probed with the CYP1A1 cDNA; that in the lower panel was probed with the actin cDNA. Results are typical of at least five separate experiments.

Figure 5 Effect of α -NF and RU486 on the induction of CYP1A1 mRNA in hepatocytes cultured in modified WME

Northern blot of total RNA (20 μ g per lane) from hepatocytes cultured for 24 h in modified WME with additions as noted. Lane 1, isolated hepatocytes before treatment; lane 2, solvent only; lane 3, 20 μ M α -NF; lane 4, 500 μ M MET; lane 5, 200 nM DEX; lane 6, 500 μ M MET plus 200 nM DEX; lane 7, 20 μ M β -NF; lane 8, 10 μ M RU486; lane 9, 10 μ M RU486 plus 500 μ M MET; lane 10, 10 μ M RU486 plus 200 nM DEX; lane 11, 10 μ M RU486 plus 20 μ M β -NF; lane 12, 10 μ M RU486 plus 500 μ M MET plus 200 nM DEX; lane 13, 20 μ M α -NF plus 20 μ M β -NF; lane 14, 20 μ M α -NF plus 500 μ M MET; lane 15, 20 μ M α -NF plus 200 nM DEX; lane 16, 20 μ M α -NF plus 500 μ M MET plus 200 nM DEX. The gel in the upper panel was probed with the CYP1A1 cDNA; that in the lower panel was probed with the actin cDNA. Results are typical of at least three separate experiments.

were treated in a balanced salt solution (HBSS + Ca/Mg) supplemented with 2.7 μ M riboflavin with or without exposure to light. In contrast, β -NF induced CYP1A1 mRNA in hepatocytes cultured in the balanced salt solution without exposure to light.

Previous experiments with continuous liver-derived cell lines demonstrated that photoactivated histidine resulted in the induction of CYP1A1-mediated aryl hydrocarbon hydroxylase activity [36,37]. However, the addition of histidine to balanced salt solutions with or without riboflavin and light treatment did not restore CYP1A1 mRNA inducibility to MET in cultured hepatocytes (results not shown). Supplementation of the balanced salt solution with riboflavin and 50 μ M tryptophan (the concentration present in WME) with minimal light illumination restored the inducibility of CYP1A1 mRNA to MET (Figure 7). Exposure of this medium to light illumination further increased the levels of CYP1A1 mRNA induction by MET (Figure 7).

Examination of the ability of metyrapone to compete with the specific binding of radiolabelled TCDD and BP to hepatic liver cytosolic protein(s)

The CYP1A1 inducer TCDD [6] has been reported to be a specific ligand for the AhR [16,17,45]. The incubation of radiolabelled TCDD with rat liver cytosolic protein results in the specific binding of protein as determined by co-incubation with excess unlabelled TCDD, although the non-specific binding to cytosolic proteins (40–50 % of total binding) is 10-fold that experienced with less hydrophobic ligands such as steroid hormone binding to steroid hormone receptors and membrane-associated binding sites [46,47]. The level of specific binding of radiolabelled TCDD with increasing concentrations of radioligand determines a K_d of 1 ± 0.3 nM for TCDD and an $[LR]_e^{max}$ of 30 ± 15.6 fmol/mg of cytosolic protein (means $\pm$ S.D. for determinations from four separate cytosol preparations). These values are in agreement with previous studies [48,49].

At a fixed concentration of radiolabelled TCDD, metyrapone significantly decreased the apparent specific levels of binding of TCDD to cytosolic protein, although within the 10–1000 μ M range of metyrapone concentrations employed, there was no concentration-dependent competition and the levels of competition did not exceed the percentage levels of non-specific binding compared with specific binding (Table 1). DEX at 100 nM did not compete with radiolabelled TCDD for binding to cytosolic protein (results not shown). Also, addition of 100 nM DEX to cytosolic fractions containing MET did not affect the apparent competition of MET for radiolabelled TCDD binding to cytosolic protein (results not shown). In contrast, the CYP1A1 inducers and AhR ligands β -NF and 3-methylcholanthrene potently competed with TCDD for binding to cytosolic protein (Table 1). The incubation of cytosolic protein with radiolabelled TCDD in the presence or absence of 100 μ M metyrapone followed by gel filtration on a Superose 12HR 10/30 FPLC column, after removal of free ligand, also indicated that metyrapone does not compete with radiolabelled TCDD for binding to a peak in radioactivity that was eluted from the column after the void peak and with high-molecular-mass cytosolic proteins, probably including AhR, which as a heteromeric complex is of the order 200–300 kDa [50,51] (results not shown). BP was relatively weak in its ability to compete with TCDD for binding to cytosolic protein and in these experiments 10 μ M BP did not result in statistically significant competition in three separate experiments (Table 1).

The incubation of radiolabelled BP with rat liver cytosolic protein also resulted in specific binding of protein as determined by co-incubation with unlabelled BP. The level of specific binding of radiolabelled BP with increasing concentrations of radioligand determined a K_d of 12 ± 5.8 nM BP and an $[LR]_e^{max}$ of 1.7 ± 0.19 pmol/mg of cytosolic protein (means $\pm$ S.D. for determinations from three separate cytosol preparations). These values

Figure 6 Effect of illumination on the induction of CYP1A1 mRNA in hepatocytes cultured in modified WME supplemented with riboflavin

Northern blot of total RNA (20 μ g per lane) from hepatocytes cultured for 24 h in modified WME supplemented with 2.7 μ M riboflavin (10-fold molar increase in concentration in standard WME) with treatments/additions as noted. Lane 1, isolated hepatocytes before treatment; lanes 2–7, with medium subjected to 30 min illumination (lane 2, solvent only; lane 3, 500 μ M MET; lane 4, 200 nM DEX; lane 5, 500 μ M MET plus 200 nM DEX; lane 6, 20 μ M β -NF; lane 7, 20 μ M β -NF plus 200 nM DEX); lanes 8–13, medium without illumination (lane 8, solvent only; lane 9, 500 μ M MET; lane 10, 200 nM DEX; lane 11, 500 μ M MET plus 200 nM DEX; lane 12, 20 μ M β -NF; lane 13, 20 μ M β -NF plus 200 nM DEX). The gel in the upper panel was probed with the CYP1A1 cDNA; that in the lower panel was probed with the actin cDNA. Results are typical of at least three separate experiments.

Figure 7 Effect of illumination and tryptophan on the induction of CYP1A1 mRNA by MET or β -NF in hepatocytes cultured in HBSS + Ca/Mg supplemented with riboflavin

(a) Northern blot of total RNA (20 μ g per lane) from hepatocytes cultured for 20 h in HBSS + Ca/Mg. Further additions were as noted. Lane 1, isolated hepatocytes before treatment; lane 2, hepatocytes cultured for 4 h without additions to culture medium; lane 3, solvent only; lane 4, 500 μ M MET; lane 5, 20 μ M β -NF; lane 6, 200 nM DEX; lane 7, 500 μ M MET plus 200 nM DEX. The gel in the upper panel was probed with CYP1A1 cDNA; that in the lower panel was probed with actin cDNA. Results are typical of three separate experiments. (b) Northern blot of total RNA (20 μ g per lane) from hepatocytes cultured for 20 h in HBSS + Ca/Mg supplemented with 2.7 μ M riboflavin with additions as noted. Lane 1, solvent only with minimal light exposure; lane 2, 500 μ M MET with minimal light exposure; lane 3, 20 μ M β -NF with minimal light exposure; lane 4, solvent only with light exposure; lane 5, 500 μ M MET with light exposure; lane 6, 20 μ M β -NF with light exposure; lane 7, 50 μ M L-tryptophan and solvent only, with minimal light exposure; lane 8, 50 μ M L-tryptophan plus 500 μ M MET with minimal light exposure; lane 9, 50 μ M L-tryptophan plus 20 μ M β -NF with minimal light exposure; lane 10, 50 μ M L-tryptophan and solvent only with light exposure; lane 11, 50 μ M L-tryptophan and 500 μ M MET with light exposure; lane 12, 50 μ M L-tryptophan plus 20 μ M β -NF with light exposure. The gel in the upper panel was probed with CYP1A1 cDNA; that in the lower panel was probed with actin cDNA. Results are typical of at least three separate experiments.

Table 1 Competition for radiolabelled TCDD and BP in binding to rat liver cytosolic protein(s)

Aliquots of cytosol were incubated with either 1 nM radiolabelled TCDD or 8 nM radiolabelled BP; non-specific binding was determined by co-incubation with 200 nM unlabelled TCDD or 4 μ M unlabelled BP respectively. Specific and non-specific bindings of radiolabel were determined at equilibrium in the presence and in the absence of the indicated concentration of unlabelled compounds. Results are typical of at least three separate experiments. * Significantly different (two-tailed) $P > 95\%$ from control specific binding, by Student's paired t test. Abbreviations: n.d., not detectable; 3MC, 3-methylcholanthrene. —, not applicable.

Unlabelled competitor	Concentration (M)	Apparent specific radiolabel binding (% of control)	
		BP	TCDD
MET	10^{-5}	$71 \pm 2.1^*$	$67 \pm 10.3^*$
MET	10^{-4}	$37 \pm 4.5^*$	$59 \pm 12.7^*$
MET	10^{-3}	$17 \pm 5.4^*$	71 ± 11.0
3MC	10^{-6}	$15 \pm 1^*$	n.d.*
β -NF	10^{-5}	$23 \pm 1.3^*$	$34 \pm 6.6^*$
BP	10^{-5}	—	84 ± 5.4
TCDD	10^{-10}	84 ± 17.3	—
TCDD	10^{-9}	75 ± 18.0	—
TCDD	10^{-8}	87 ± 18.8	—
TCDD	10^{-7}	78 ± 11.3	—

are in agreement with previous studies [18,52]. By employing a fixed concentration of radiolabelled BP, it can be seen that metyrapone significantly reduced the levels of binding of BP to cytosolic protein in a concentration-dependent manner and in excess of any effects on non-specific binding of BP (Table 1). β -NF and 3-methylcholanthrene competed with BP for binding to cytosolic protein; in contrast, no significant competition was observed with up to 100 nM TCDD (Table 1).

DISCUSSION

The results presented here indicate that the expression of hepatic CYP1A1 mRNA is induced in rats treated with MET in the presence of a glucocorticoid. Induction by MET/DEX in hepatocytes cultured in a fully defined serum-free medium indicate that MET and DEX act directly on the liver *in vivo* to effect CYP1A1 mRNA induction. The ability of cycloheximide to substitute for DEX *in vitro* suggests that CYP1A1 mRNA induction has an obligatory dependence on the presence of MET.

The characteristics of MET/DEX-associated CYP1A1 mRNA induction differ from those of β -NF *in vitro* in the time course for detectable mRNA induction, the nature of the dependence on glucocorticoid, and the requirement for a photoactivated medium constituent. These observations indicate that the mechanism of induction of CYP1A1 mRNA by MET/DEX is different from that of induction by β -NF, which has been established to be mediated by a direct interaction with the AhR [40].

Time course

The time course for the increase in the detectable levels of CYP1A1 mRNA *in vitro* is slower in hepatocytes treated with DEX/MET than in those treated with β -NF. The rapid effects of both MET and DEX on CYP3A expression [23,53] suggest that this delay is not associated with a difference in cellular uptake but is due to a time-consuming process critical to induction.

Role of glucocorticoid

The induction of CYP1A1 mRNA by β -NF is enhanced by DEX, although DEX alone does not induce CYP1A1 mRNA. The role of DEX in the MET-associated induction of CYP1A1 mRNA in hepatocytes cultured in modified WME therefore seems to be different in that it is absolutely required. Examination of the concentration-dependent effects of DEX on the β -NF- and MET-associated induction of CYP1A1 mRNA shows that the concentration of DEX required to have approximately half-maximal induction is in the nanomolar range for both compounds. This concentration is approximately in the range of the K_d for DEX for the rat GR in rat hepatic cytosol [46], a concentration relevant to the effects observed because this synthetic steroid is several orders more potent than other glucocorticoids such as hydrocortisone at inducing GR-dependent genes in the liver, presumably because DEX is relatively slowly metabolized to non-GR agonist metabolites [21,35]. In addition, the levels of induction of CYP1A1 mRNA induced by MET/DEX are decreased when hepatocytes are additionally treated with the GR antagonist RU486. The role of DEX in the MET/DEX-associated induction of CYP1A1 mRNA is therefore likely to be mediated through the GR. The role of glucocorticoids in the mechanism of induction of CYP1A1 has been established for classic AhR-dependent inducers as being associated with an enhancement (approx. 2–4-fold) of CYP1A1 gene transcription through the interaction of ligand-activated GR with glucocorticoid response elements (GREs) present within the first intron of the CYP1A1 gene [54–56]. DEX is therefore likely to increase the transcription of CYP1A1 mRNA associated with either β -NF or MET by this mechanism. However, because cycloheximide substitutes for DEX in MET-associated induction of CYP1A1 mRNA, GR activation might also inhibit the activity of a constitutively expressed factor that down-regulates CYP1A1 gene transcription. In this respect it has been demonstrated that the presence of a region –843 to –746 bp 5' upstream of the rat CYP1A1 gene suppresses basal CYP1A1 promoter activity and it is termed the CYP1A1 negative regulatory element (NRE) [57,58]. The GR might therefore interact with protein(s) that bind to the CYP1A1 NRE and such an interaction might be critical to effect the CYP1A1 induction associated with MET. Alternatively, the GR might repress the expression of the negative acting factor or induce the expression of factors that repress the expression or function of the negative-acting factor of CYP1A1 gene expression.

Medium constituent

An examination of the effect of MET/DEX and β -NF on the induction of CYP1A1 mRNA in hepatocytes cultured in a balanced salt solution indicates that MET/DEX induction requires the presence of a medium constituent *in vitro*, in contrast with that of β -NF. Binding results indicate that MET does not compete with TCDD for binding to cytosolic protein(s), in contrast with β -NF, suggesting that MET is not a ligand for the AhR. However, MET and β -NF both compete with the CYP1A1 inducer BP for binding to cytosolic protein(s), suggesting that these compounds bind to the 4 S PAH-binding protein. In our studies, BP was found to be a CYP1A1 mRNA inducer (results not shown), in agreement with others [59,60], yet BP is reported to be a weak ligand of the AhR [61], in agreement with results presented here. Bresnick and co-workers have suggested that the 4 S PAH-binding site might mediate the induction of CYP1A1 by compounds such as BP [62]. However, the fact that MET and DEX fail to induce CYP1A1 mRNA in hepatocytes cultured in a balanced salt solution suggests, with

respect to MET-associated CYP1A1 induction, that MET interaction with the 4 S PAH-binding protein does not alone mediate CYP1A1 mRNA induction. The effect of medium photoactivation on increasing CYP1A1 mRNA induction by MET/DEX and the restoration of induction of CYP1A1 mRNA by MET in hepatocytes cultured in a balanced salt solution containing photoactivated tryptophan indicate that the MET-associated induction of CYP1A1 mRNA is dependent, at least *in vitro*, on the generation of photoactivated products of tryptophan. Previous work by this group [36,37] and others [63–65] has shown that culture medium amino acid photoactivation results in the generation of CYP1A1 inducers, most notably from histidine and tryptophan. In experiments reported here, tryptophan, but not histidine, restores the induction of CYP1A1 mRNA in hepatocytes treated with MET in a balanced salt solution when subjected to photoactivation. Recent work in mouse cell lines has demonstrated that photo-oxidation products of tryptophan, which have been shown to bind to the AhR [66], induce CYP1A1 gene expression, an effect dependent on the expression of AhR and AhR transformation to a xenobiotic response element-binding form in gel mobility-shift assays [65]. The requirement for photoactivated products of tryptophan for the induction of CYP1A1 mRNA by MET *in vitro* suggests that MET indirectly induces CYP1A1 gene expression *in vitro* by affecting the levels of tryptophan-photoactivated products. The variability in the levels of CYP1A1 mRNA induced by MET/DEX compared with those by β -NF observed in these studies is probably associated with the variability in exposure of culture medium to light between experiments. It is possible that tryptophan oxidation products are functional endogenous ligands for the AhR and are degraded by CYPs to inactive inducers. Thus metyrapone might increase their intracellular levels by inhibiting their degradation by CYP.

These results show that MET in the presence of either DEX or cycloheximide induces hepatic CYP1A1 mRNA expression. Receptor–ligand binding analyses suggest that MET does not bind to the AhR. The requirement for photoactivated tryptophan products *in vitro*, which are known to bind to the AhR, in MET-associated induction of CYP1A1 suggests that MET acts to elevate the levels of photoactivated tryptophan products. The ability of MET to elevate the levels of a photoactivated amino acid inducer *in vitro* suggests that the induction of CYP1A1 associated with MET is mediated by a similar mechanism *in vivo* and therefore that there is an endogenously biosynthesized amino acid-derived ligand for the AhR. The requirement for glucocorticoid or cycloheximide in the MET-associated induction of CYP1A1 mRNA suggests that derepression by a GR-modulated negative-acting factor of gene expression might be critical to induction by this mechanism.

This work was funded in part by a grant from the University of London Central Research Fund.

REFERENCES

- Nelson, D. R., Koymans, L., Kamataki, T., Stegeman, J. J., Feyereisen, R., Waxman, D. J., Waterman, M. R., Gotoh, O., Coon, M. J., Estabrook, R. W. et al. (1996) *Pharmacogenetics* **6**, 1–42
- Gonzalez, F. J. (1990) *Pharmacol. Therap.* **45**, 1–38
- Okey, A. B., Riddick, D. S. and Harper, P. A. (1994) *Trends Pharmacol. Sci.* **15**, 226–232
- Ema, M., Sogawa, K., Watanabe, N., Chujoh, Y., Matsushita, N., Gotoh, O., Funae, Y. and Fujii-Kuriyama, Y. (1992) *Biochem. Biophys. Res. Commun.* **184**, 246–253
- Carver, L. A., Hogenesch, J. B. and Bradfield, C. A. (1994) *Nucleic Acids Res.* **22**, 3038–3044
- Whitlock, J. P. (1993) *Chem. Res. Toxicol.* **6**, 754–763
- Perdew, G. H. (1988) *J. Biol. Chem.* **263**, 13802–13805
- Chen, H. S. and Perdew, G. H. (1994) *J. Biol. Chem.* **269**, 27554–27558
- Hoffman, E. C., Reyes, H., Chu, F. F., Sander, F., Conley, L. H., Brooks, B. A. and Hankinson, O. (1991) *Science* **252**, 954–958
- Sogawa, K., Nakano, R., Kobayashi, A., Kikuchi, Y., Ohe, N., Matsushita, N. and Fujii-Kuriyama, Y. (1995) *Proc. Natl. Acad. Sci. U.S.A.* **92**, 1936–1940
- Fischer, J. M., Denison, M. S. and Whitlock, J. P. (1988) *J. Biol. Chem.* **265**, 9676–9681
- Denison, M. S., Fisher, J. M. and Whitlock, J. P. (1988) *J. Biol. Chem.* **265**, 17221–17224
- Hankinson, O. (1995) *Annu. Rev. Pharmacol. Toxicol.* **35**, 307–340
- Fernandez-Salguero, P., Pineau, T., Hilbert, D. M., McPhail, T., Lee, S. S. T., Kimura, S., Nebert, D. W., Rudikoff, S., Ward, J. M. and Gonzalez, F. J. (1995) *Science* **268**, 722–726
- Raha, A., Joyce, T., Gusky, S. and Bresnick, E. (1995) *Arch. Biochem. Biophys.* **322**, 395–404
- Houser, W. H., Zielinski, R. and Bresnick, E. (1986) *Arch. Biochem. Biophys.* **251**, 361–368
- Raha, A., Reddy, V., Houser, W. and Bresnick, E. (1990) *J. Toxicol. Environ. Health* **29**, 339–355
- Raha, A., Wagner, C., MacDonald, R. G. and Bresnick, E. (1994) *J. Biol. Chem.* **269**, 5750–5756
- Ogawa, H., Gomi, T., Imamura, T., Kobayashi, M. and Huh, N. (1997) *Biochem. Biophys. Res. Commun.* **233**, 300–304
- Shean, K. and Paine, A. J. (1990) *Biochem. J.* **267**, 715–717
- Wright, M. C., Paine, A. J., Skett, P. and Auld, R. (1994) *J. Steroid Biochem. Mol. Biol.* **48**, 271–276
- Paine, A. J. (1990) *Chem–Biol. Interact.* **74**, 1–31
- Wright, M. C., Wang, X.-J., Pimenta, M., Ribeiro, V., Paine, A. J. and Lechner, M. C. (1996) *Mol. Pharmacol.* **50**, 856–863
- Wright, M. C. (1994) Ph.D. Thesis, University of London
- Goodwin, B., Liddle, C., Murray, M., Tapner, M., Rooney, T. and Farrell, G. C. (1996) *Biochem. Pharmacol.* **52**, 219–227
- Brown, S. E. S., Quattrochi, L. C. and Guzelian, P. S. (1997) *Arch. Biochem. Biophys.* **342**, 134–142
- Padgham, C. R. W. and Paine, A. J. (1993) *Biochem. J.* **289**, 621–624
- Padgham, C. R. W., Paine, A. J., Phillips, I. R. and Shephard, E. A. (1992) *Biochem. J.* **285**, 929–932
- Ledirac, N., Delescluse, C., de Sousa, G., Pralavorio, M., Lesca, P., Amichot, M., Berge, J. B. and Rahman, R. (1997) *Toxicol. Appl. Pharmacol.* **144**, 177–182
- Washburn, B. S., Rein, K. S., Baden, D. G., Walsh, P. J. and Hinton, D. E. (1997) *Arch. Biochem. Biophys.* **343**, 149–156
- Jeong, H. G., Lee, S. S., Kim, H. K. and Yang, K. H. (1977) *Arch. Biochem. Biophys.* **233**, 619–622
- Dzeletovic, N., McGuire, J., Daujat, M., Tholander, J., Ema, M., Fujii-Kuriyama, Y., Bergman, J., Maurel, P. and Poellinger, L. (1997) *J. Biol. Chem.* **272**, 12705–12713
- Usansky, J. I. and Damani, L. A. (1992) *Drug Metab. Dispos.* **20**, 64–69
- Fagan, J. B., Pastewka, J. V., Chalberg, S. C., Gozokara, E., Guengerich, F. P. and Gelboin, H. V. (1986) *Arch. Biochem. Biophys.* **244**, 261–272
- Wright, M. C. and Paine, A. J. (1992) *Biochem. Pharmacol.* **43**, 237–243
- Paine, A. J. and Francis, J. E. (1980) *Chem–Biol. Interact.* **30**, 343–353
- Paine, A. J. (1976) *Biochem. J.* **158**, 109–117
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Leatherbarrow, R. J. (1987) *Enzfitter*, Elsevier Biosoft, Cambridge
- Morville, A. L., Thomas, P., Levin, W., Reik, L., Ryan, D. E., Raphael, C. and Adesnick, M. (1983) *J. Biol. Chem.* **258**, 3901–3906
- Blaauboer, B. J. and Paine, A. J. (1979) *Biochem. Biophys. Res. Commun.* **90**, 368–374
- Baulieu, E. E. (1989) *Science* **245**, 1351–1357
- Merchant, M. and Safe, S. (1995) *Biochem. Pharmacol.* **50**, 663–668
- Gasiewicz, T. A., Kende, A. S., Rucci, G., Whitney, B. and Willey, J. J. (1996) *Biochem. Pharmacol.* **52**, 1787–1803
- Lesca, P., Fernandez, N. and Roy, M. (1987) *J. Biol. Chem.* **262**, 4827–4835
- Wright, M. C. and Paine, A. J. (1994) *Biochem. Biophys. Res. Commun.* **201**, 973–979
- Wright, M. C. and Paine, A. J. (1994) in *Cytochrome P450*, 8th International Conference (Lechner, M. C., ed.), pp. 733–736, John Libbey Eurotext, Paris
- Gasiewicz, T. A. and Neal, R. A. (1982) *Anal. Biochem.* **124**, 1–11
- Denison, M. S., Fine, J. and Wilkinson, C. F. (1984) *Anal. Biochem.* **142**, 28–36
- Poland, A. and Glover, E. (1987) *Biochem. Biophys. Res. Commun.* **261**, 1439–1449
- Landers, J. P. and Bunce, N. J. (1991) *Biochem. J.* **276**, 273–287
- Roy, M., Fernandez, N. and Lesca, P. (1988) *Eur. J. Biochem.* **172**, 593–599
- Pereira, T. M. and Lechner, M. C. (1995) *Eur. J. Biochem.* **229**, 171–177
- Mathis, J. M., Houser, W. H., Bresnick, E., Cidlowski, J. A., Hines, R. N., Prough, R. A. and Simpson, E. R. (1989) *Arch. Biochem. Biophys.* **269**, 93–105

-
- 55 Linder, M. W. and Prough, R. A. (1993) Arch. Biochem. Biophys. **302**, 92–102
- 56 Prough, R. A., Linder, M. W., Pinaire, J. A., Xiao, G. H. and Falkner, F. C. (1996) FASEB J. **10**, 1369–1377
- 57 Sterling, K., Weaver, J., Ho, L. C. and Bresnick, E. (1993) Mol. Pharmacol. **44**, 560–568
- 58 Sterling, K. and Bresnick, E. (1996) Mol. Pharmacol. (1996) **49**, 329–337
- 59 Tierney, B., Weaver, D., Heintz, W., Scaeffler, W. I. and Bresnick, E. (1980) Arch. Biochem. Biophys. **200**, 513–523
- 60 Zytkevich, T. H. (1982) Cancer Res. **42**, 4387–4393
- 61 Raha, A., Reddy, V., Houser, W. H. and Bresnick, E. (1990) J. Toxicol. Environ. Health **29**, 339–355
- 62 Bhat, R., Weaver, J. A., Wagner, J. A., Bodwell, J. E. and Bresnick, E. (1996) J. Biol. Chem. **271**, 32551–32556
- 63 Rannug, A. and Rannug, U. (1989) Chemosphere **18**, 1101–1106
- 64 Kocarek, T. A., Shuetz, E. G. and Guzelian, P. S. (1993) In Vitro Cell Dev. Biol. **29A**, 62–66
- 65 Sindhu, R. K., Reisz-Porszasz, S., Hankinson, O. and Kikkawa, Y. (1996) Biochem. Pharmacol. **52**, 1883–1893
- 66 Rannug, A., Rannug, U., Rosenkranz, H. S., Wingvist, L., Westerholm, R., Agurell, E. and Grafstrom, A. (1987) J. Biol. Chem. **262**, 15422–15427
- 67 Wang, X.-J., Hodgkinson, C., Wright, M. C. and Paine, A. J. (1997) Biochem. J. **328**, 937–944

Received 23 October 1997/17 December 1997; accepted 9 January 1998