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Glucocorticoid Regulation of Polycyclic Aromatic Hydrocarbon Induction of Cytochrome P450IA1, Glutathione S-Transferases, and NAD(P)H:Quinone Oxidoreductase in Cultured Fetal Rat Hepatocytes

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SUMMARY

The regulation of polycyclic aromatic hydrocarbon-inducible enzymes, cytochrome P450IA1, NAD(P)H:quinone oxidoreductase, and glutathione *S*-transferases, by glucocorticoids was investigated using primary fetal rat hepatocyte culture. Treatment of cells in culture with 1,2-benzanthracene (100 μM, 72 hr) resulted in 60-, 2-, and 6-fold increases in cytochrome P450IA1, glutathione *S*-transferase, and NAD(P)H:quinone reductase activities, respectively. The inductive effect of 1,2-benzanthracene on cytochrome P450IA1 and glutathione *S*-transferase (1-chloro-2,4-dinitrobenzene conjugation) activities was potentiated approximately 3- and 2- to 3-fold, respectively, when dexamethasone (0.01–1 μM) was included in the culture medium. In contrast, 1 μM dexamethasone was found not to potentiate the induction of NAD(P)H:quinone oxidoreductase activity by 1,2-benzanthracene. Treatment of cultured hepatocytes with dexamethasone alone, at concentrations of up to 100 μM, resulted in a 2- to 4-fold increase in glutathione *S*-transferase and NAD(P)H:quinone oxidoreductase activity. Both the induction of glutathione *S*-transferase activity by high concentrations of dexamethasone alone and the potentiation of 1,2-benzanthracene induction by lower concentrations of dexamethasone were observed for other steroids of the glucocorticoid class in conjunction with a variety of polycyclic aromatic hydrocarbons. Western immunoblot analyses indicated that low concentrations of dexamethasone (0.1–1 μM) potentiated 1,2-benzanthracene-dependent induction of cytochrome P450IA1, glutathione *S*-transferase Ya/Yc subunit, and NAD(P)H:quinone oxidoreductase content. Additionally, increased glutathione *S*-transferase activity in response to concentrations of dexamethasone exceeding 1 μM was associated with concomitant increases in Ya/Yc and Yb subunit content. Potentiation of polycyclic aromatic hydrocarbon induction of cytochrome P450IA1, glutathione *S*-transferase, and NAD(P)H:quinone oxidoreductase protein content by low concentrations of glucocorticoids and induction of glutathione *S*-transferase and NAD(P)H:quinone oxidoreductase by high concentrations of glucocorticoids alone indicates the importance of these endogenous compounds in the regulation of some hepatic enzymes involved in xenobiotic metabolism.

Adrenal corticosteroids (glucocorticoids) and their potent synthetic analogs (notably DEX) have been shown to modulate the expression of a diverse array of hepatic drug-metabolizing enzyme systems, including cytochrome P450IIIA1 (P-450p) (1, 2), cytochrome P450IIB1 (P-450b), NADPH-cytochrome P450 reductase (3), UDP-glucuronosyltransferase (4), and γ-glutamyl transpeptidase (5–7). Glucocorticoids alone, at concentrations that have little effect in altering P450IA1 activity and content, have also been shown to potentiate PAH induction of P450IA1 *in vitro* (8, 9). Using adrenalectomized male rats, we have demonstrated that coadministration of 3-methylcholanthrene and DEX *in vivo* enhances the PAH-dependent induction of P450IA1 by 2.7-fold, relative to administration of the PAH alone (10). This result demonstrates that, under conditions of extremely low circulating levels of glucocorticoids *in vivo*, the response of P450IA1 expression due to PAH is diminished relative to animals possessing higher levels of circulating glucocorticoids. The increases in P450IA1 activity and protein were shown to be due to increased levels of mRNA specific for P450IA1 and increased *de novo* synthesis of the hemoprotein (8, 9). Glucocorticoid regulatory elements, located within intron I of the P450IA1 rat gene, are apparently responsible for the enhanced transcriptional activation of the P450IA1 gene by PAH after addition of glucocorticoids to cultured cell systems (11, 12).

GSTs (EC 2.5.1.18) and QR (EC 1.6.99.2) are two distinct

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ABBREVIATIONS: DEX, dexamethasone; PAH, polycyclic aromatic hydrocarbons; BA, 1,2-benzanthracene; CC, corticosterone; CDNB, 1-chloro-2,4-dinitrobenzene; GST, glutathione *S*-transferase; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide; P450IA1, cytochrome P450IA1; PCN, pregnenolone-16α-carbonitrile; QR, NAD(P)H:quinone oxidoreductase.

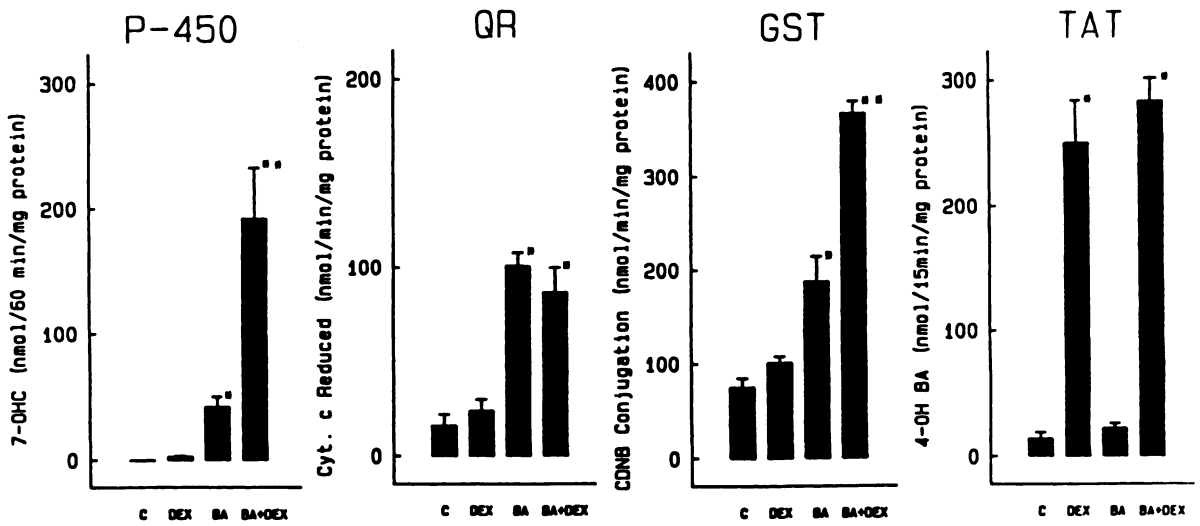

Fig. 1. Effect of DEX on the induction of P450IA1 (*P450*), QR, GST, and tyrosine aminotransferase (*TAT*) activities of cultured fetal rat hepatocytes by BA. Cells were initially cultured in the absence of DEX. After 24 hr in culture, medium was replaced with fresh medium (control) or with medium containing DEX (1 μM for P-450 and QR and 10 nM for GST and tyrosine aminotransferase), BA (100 μM), or BA (100 μM) plus DEX (1 μM for P-450 and QR and 10 nM for GST and tyrosine aminotransferase). The values represent the mean ± standard error of data from three or four replicate dishes obtained after 96 hr in culture. *Significantly different from control cells ($p < 0.05$). **Significantly different from BA-treated cells ($p < 0.05$).

TABLE 1

Rates of MTT reduction of control, DEX-, BA-, or BA plus DEX-treated fetal rat liver cells

Cells were seeded into 12-well plates and sets of wells (three wells/treatment) were treated with BA and/or DEX for 72 hr as described in Experimental Procedures. The first set of wells were assayed for QR activity in the presence of menadione, the second set was assayed in the presence of menadione and dicoumarol (0.6 mM), and the third set was assayed in the absence of menadione, as described by Prochaska and Santamaria (21). The fourth set of wells was assayed for protein content. The values reported are expressed as the mean of nmol of MTT reduced/15 min/mg of cell protein, ± standard error.

Treatment of cells	MTT reduction		
	Standard assay	Standard assay in the presence of dicoumarol	Standard assay without menadione
		nmol/15 min/mg	
Control	89 ± 14	32 ± 5	19 ± 5
DEX (1 μM)	108 ± 6	32 ± 6	34 ± 6
BA (100 μM)	290 ± 31	42 ± 8	54 ± 13
BA (100 μM) + DEX (1 μM)	249 ± 16	39 ± 7	55 ± 10

enzyme systems involved in xenobiotic detoxication that are responsive to PAH. GSTs, present in fetal human (13, 14) and rat liver (15), catalyze the conjugation of glutathione to various electrophilic compounds (for reviews see Refs. 16 and 17). Rat liver GSTs are homo- and hetero-dimers composed of Ya, Yb, and Yc subunits, which apparently are all inducible by PAH (16). QR (DT-diaphorase), which catalyzes the two-electron reduction of quinones to form the respective hydroquinone, is believed to confer protection against semiquinone radical cytotoxicity (for review see Ref. 18). In contrast to the GSTs, the regulation of QR is less well defined. Because P450IA1 induction by PAH is strikingly potentiated by DEX (8–12), we investigated the role of glucocorticoids in regulating BA-dependent induction of GSTs and QR, using primary cultures of fetal rat hepatocytes.

Experimental Procedures

Materials. Collagenase (Type I) was obtained from Worthington Biochemicals (Freehold, NJ). Hyaluronidase (Type II), insulin-transferrin-sodium selenite medium supplement, L-ornithine, MTT, digitonin, menadione, dicoumarol, glucose-6-phosphate, yeast glucose-6-phosphate dehydrogenase, riboflavin, BA, and the various steroid hormones were obtained from Sigma Chemical Co. (St. Louis, MO). Arginine-free Eagle's minimum essential medium and antibiotic/antimycotic solutions were purchased from GIBCO (Grand Island, NY). Purified P450IA1 was kindly provided by Dr. F. P. Guengerich (Vanderbilt University School of Medicine, Nashville, TN). Rabbit anti-rat polyclonal immunoglobulins were raised against P450IA1 in our laboratory as previously described (19). Rabbit serum containing immunoglobulins against rat hepatic GST Ya/Yc and Yb subunits and against QR were generously provided by Dr. C. B. Pickett. Protein A (^{125}I-labeled) was purchased from New England Nuclear (Boston, MA).

Isolation of fetal hepatocytes. Pregnant Sprague-Dawley rats (Hsd:Sprague-Dawley, 13 to 16 days of gestation from Harlan Sprague-Dawley Inc., Indianapolis, IN) were killed at 16 to 21 days of gestation by cervical dislocation after CO_2 anesthesia. After aseptic removal from the uterus, rat fetuses were killed by decapitation. The fetal livers were removed, washed, and minced in Dulbecco's phosphate-buffered saline solution. Hepatocytes were prepared by collagenase/hyaluronidase digestion using the method of Fry *et al.* (20). Cells were counted and viability was determined by Trypan blue exclusion. Cell yields were on the order of 10×10^6 cells/pup liver and cell viability was 92 ± 4% (six experiments).

Primary culture of fetal hepatocytes. Hepatocytes were suspended in arginine-free Eagle's minimum essential medium supplemented with L-ornithine (0.6 μM), insulin-transferrin-sodium selenite medium supplement (5 μg/ml, 5 μg/ml, and 5 ng/ml, respectively), penicillin (100 units/ml), streptomycin (100 μg/ml), kanamycin sulfate (100 μg/ml), fungizone (2.5 μg/ml), and mycostatin (100 μg/ml). Cells (2×10^6 in 2 ml) were seeded in 35-mm collagen-coated tissue culture dishes and incubated in an atmosphere of humidified air (95%) and CO_2 (5%), as previously described (9). For the measurement of QR activity of cells *in situ*, cells were seeded in 22-mm collagen-coated 12-well plates containing 1.5×10^6 cells in 1.5 ml of medium (21). After 24 hr, the medium in all dishes was replaced with fresh medium.

Induction of drug-metabolizing enzymes. To initiate induction of the enzymes studied, aliquots of concentrated (100×) stock solutions of PAH in acetone were added to the fresh medium in the culture dishes. Similarly, aliquots of concentrated (100×) stock solutions of various steroid hormones in ethanol were added to obtain the indicated concentrations in the medium. Equivalent amounts of solvent added

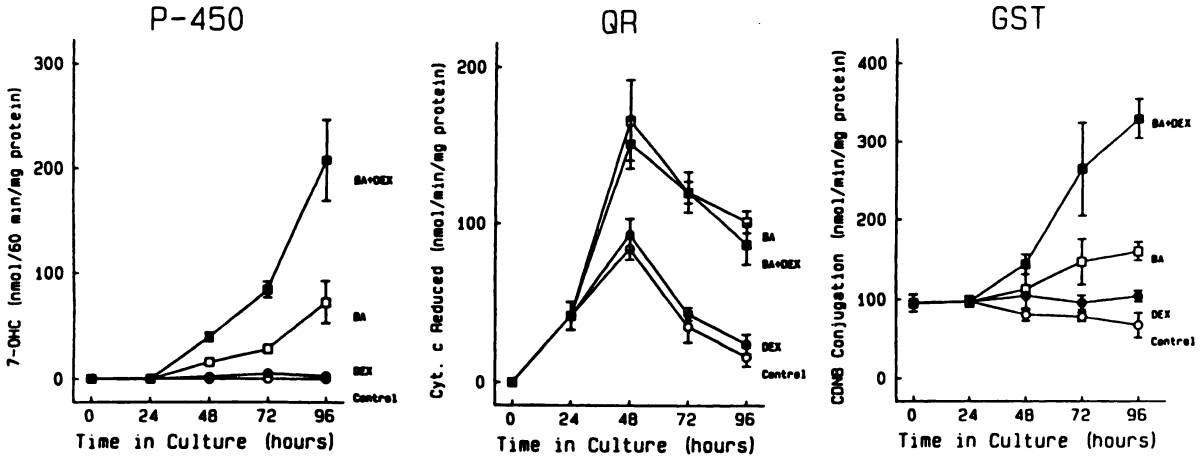

Fig. 2. Time course of induction of P450IA1 (*P450*), QR, and GST activities of cultured fetal rat hepatocytes by BA in the absence and presence of DEX. Cells were initially cultured in the absence of DEX. After 24 hr in culture, the medium was replaced with fresh medium (control) (○) or with medium containing DEX (●) (1 μM for P-450 and QR and 10 nM for GST), BA (□) (100 μM), or BA plus DEX (■). Activities of P-450, QR, and GST were determined at 24, 48, 72, or 96 hr. Each *point* represents the mean ± standard error of data obtained from three or four replicate dishes.

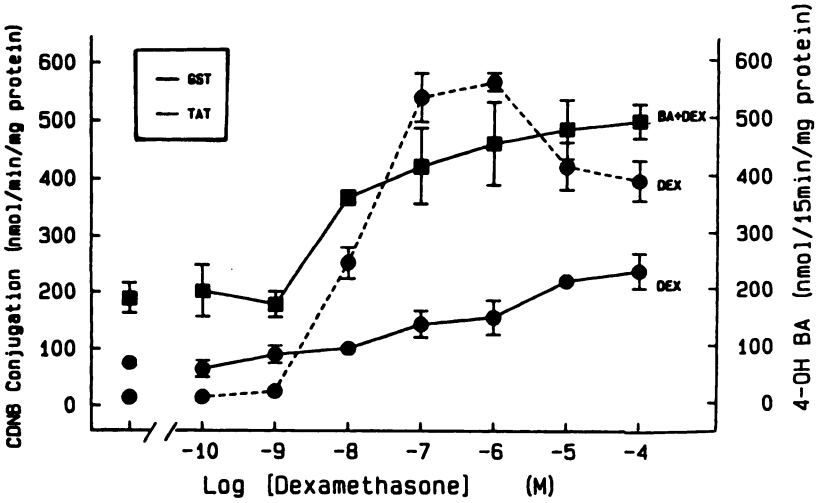

Fig. 3. Concentration-response relation for the induction of GST activities by DEX in cultured fetal rat hepatocytes in the absence or presence of BA. Cells were initially cultured in the absence of DEX. After 24 hr in culture, the medium was replaced and cells were maintained for 72 hr in the presence of various concentrations of DEX in the absence (●) or presence of BA (■) (100 μM) for GST (——) or tyrosine aminotransferase (- - -). Each *point* represents the mean ± standard error of data obtained from four replicate dishes.

to control cells were not found to significantly alter cell viability or enzyme activity.

Enzyme assays. P450IA1 activity was measured in cell monolayers using the substrate 7-ethoxycoumarin, as described by Wiebkin *et al.* (22), and QR activity was measured in cells *in situ*, as described by Prochaska and Santamaria (21). A molar absorptivity for reduced MTT of 11,300 M^{-1} cm^{-1} was used to quantitate MTT reduction. For the remaining assays, cell monolayers were washed with phosphate-buffered saline, recovered after lysis in the appropriate cell harvest buffer (1 M potassium phosphate buffer at pH 6.5 for GST or pH 7.4 for QR), and centrifuged (15,000 × *g* for 10 min). The supernatant was assayed for enzyme activity, as well as for Western immunoblot analysis. GST activity was measured using the substrate CDNB, as described by Habig *et al.* (23). QR activity was also measured in cell supernatants by monitoring the reduction of cytochrome *c* in the absence and presence of dicoumarol (10 μM), as described by Ernster (24). Tyrosine aminotransferase activity was measured by monitoring the formation of 4-hydroxybenzaldehyde from L-tyrosine and α-ketoglutarate, as described by Granner and Tomkins (25). Protein concentration was determined by the method of Lowry *et al.* (26).

Immunoelectrophoretic (Western immunoblot) analysis. Cells were harvested in phosphate-buffered saline containing cholate (1%), sodium dodecyl sulfate (0.1%), and phenylmethylsulfonyl fluoride (0.1 mM), as previously described (9). Equivalent amounts of cellular proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10% polyacrylamide) and were transferred electrophoretically to nitrocellulose sheets, which were developed according to the method of Mendelson *et al.* (27) including incubation with bovine serum albumin (Fraction V). The nitrocellulose paper was incubated with rabbit anti-rat serum containing immunoglobulins raised against either P450IA1, GST Ya/Yc and Yb subunits, or QR. The nitrocellulose sheets were then incubated with protein A (^{125}I-labeled) and visualized by autoradiography using Kodak X-Omat-AR film. Quantitation of Western immunoblots was accomplished with a Bio-Rad model 300 videodensitometer. The measurements were made in the linear range of the instrument's response.

Results

Glucocorticoid potentiation of PAH induction of P450IA1, GST, and QR in cultured fetal rat hepatocytes. P450IA1, GST, and QR activities of fetal rat hepatocytes in culture were induced 60-, 2-, and 4-fold, respectively, upon exposure to BA (100 μM) for 72 hr (Fig. 1). DEX further potentiated the BA-dependent induction of P450IA1 3-4-fold and GST 2-fold in fetal hepatocytes cultured for 72 hr. This concentration of DEX (1 μM and 10 nM, respectively) had little or no effect alone on P450IA1 or GST activity. In contrast, 1 μM DEX was found not to potentiate the induction of QR activity by BA measured either in cell supernatant fractions (Fig. 1) or in intact cells (Table 1). As expected, tyrosine

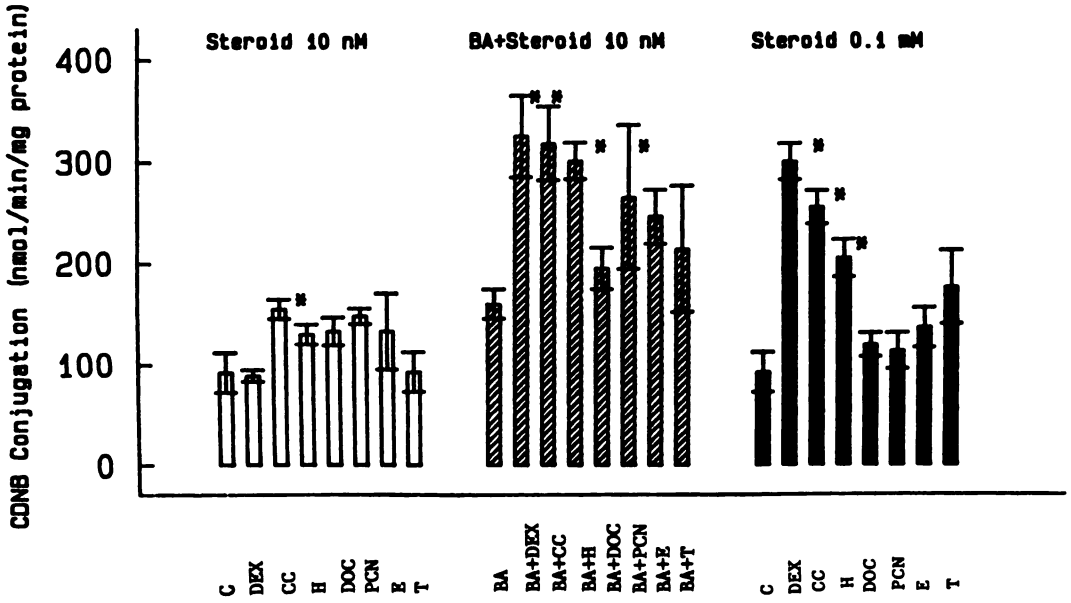

Fig. 4. Effect of various steroids on the induction of GST activity of cultured fetal rat hepatocytes in the absence or presence of BA. Cells were cultured in the absence of DEX. After 24 hr in culture, the medium was replaced and cells were maintained for 72 hr in the presence of low concentrations of steroids (*Steroid 10 nM*) or in the presence of low concentrations of steroid (10 nM) plus BA (100 μM) (*BA + Steroid*). Additionally, cells were also maintained in the presence of high concentrations of the various steroids (*Steroid 0.1 mM*). The steroids examined were DEX, CC, hydrocortisone (*H*), deoxycorticosterone (*DOC*), PCN, 17β-estradiol (*E*), and testosterone (*T*). *C*, Cells not treated with steroid. Each value represents the mean ± standard error of data obtained from four replicate dishes. *Significantly different from control group ($p < 0.05$).

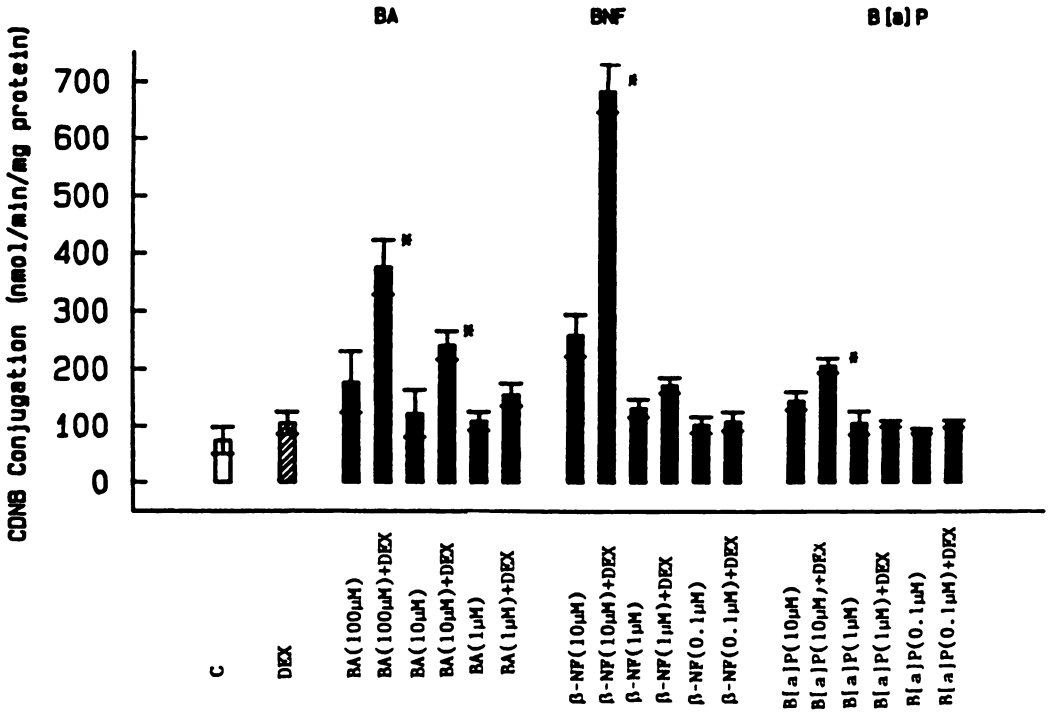

Fig. 5. Effect of various PAH on the induction of GST activity of cultured fetal rat hepatocytes in the absence or presence of DEX. Cells were maintained for 72 hr in the absence (*C*) (control cells) or presence of BA (100 to 1 μM), β-naphthoflavone (10 to 0.1 μM), or benzo[*a*]pyrene (10 to 0.1 μM) (B[*a*]P) in the absence or presence of DEX (10 nM). Each value represents the mean ± standard error of data from three or four replicate dishes of cells maintained for 72 hr in culture in the presence of PAH and steroid. *Significantly different from the PAH alone ($p < 0.05$).

aminotransferase activity was increased by DEX or BA plus DEX but not by BA alone.

Temporal profiles for DEX potentiation of BA induction of cellular P450IA1 and GST activities were similar (Fig. 2). The induction of QR activity by BA was maximal at or before 48 hr and decreased at later times; no DEX potentiation was observed. Additionally, the DEX concentration-response profiles for the potentiation of BA induction of GST activity (EC_{50}, ~10–50 nM) were similar to that found for tyrosine aminotransferase activity (EC_{50}, 10 nM), a marker for hepatic glucocorticoid action (Fig. 3). DEX alone elevated GST activity with a different concentration dependence than the response observed for tyrosine aminotransferase. Maximal induction of GST activity was not observed below a concentration of 100 μM DEX alone.

In characterizing the structure-function relationship for the potentiated induction of GST activity, we exposed the cultured hepatocytes to various steroid and PAH derivatives. The glucocorticoids DEX, CC, and hydrocortisone all potentiated BA induction of GST activity approximately 2-fold, compared with solvent controls (Fig. 4). The antiglucocorticoid PCN also potentiated BA induction of GST activity, but the activities

TABLE 2

Relative induction of P450IA1, GST subunit Ya, GST subunit Yb, and QR measured by densitometric analyses of Western immunoblots

The values given are the relative densities of the protein of interest on an autoradiogram relative to the band seen with cellular proteins from untreated or control cells.

Cell treatment	Relative density			
	P450IA1	GST Ya subunit	GST Yb subunit	QR
Control	1.0	1.0	1.0	1.0
DEX (1 μM)	4.0	1.9	1.2	1.0
DEX (100 μM)	3.0	8.8	8.8	58.
BA (100 μM)	52.	16.	1.2	81.
BA + DEX (1 μM)	71.	28.	1.0	200.

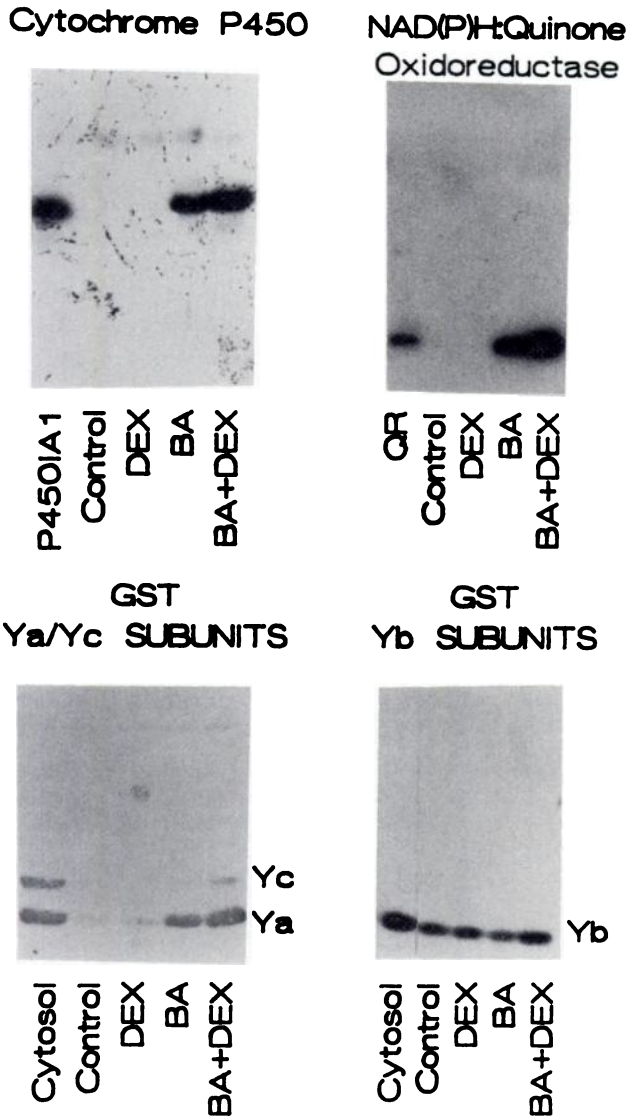

Fig. 6. Western immunoblot analyses of P450IA1, QR, and GST Ya, Yb, and Yc subunit content of fetal rat hepatocytes maintained in culture in the presence of BA and low concentrations of DEX. The *first lane* of each *panel* is a standard of either pure protein (P450IA1, 25 ng; QR, 300 ng) or liver cytosol (*Cytosol*) (5 μg of protein) obtained from male Sprague-Dawley rats treated with 3-methylcholanthrene. Cell protein (10 μg) was from fetal rat hepatocytes maintained in culture for 72 hr in the absence of inducing agents (control), or in the presence of 1 μM DEX (*DEX*), 100 μM BA (*BA*), or 100 μM BA plus 1 μM DEX (*BA + DEX*).

were more variable in the presence of PCN than the other glucocorticoids. In contrast, deoxycorticosterone, a precursor of CC, and other classes of steroid hormones examined failed to potentiate induction of GST activity by BA. Likewise, the PAH BA, β-naphthoflavone, and benzo[*a*]pyrene induced GST activity of fetal hepatocytes in a dose-dependent manner and DEX potentiated the induction of GST activity only at the higher concentrations of PAH (Fig. 5). Concentrations of benzo[*a*]pyrene and β-naphthoflavone exceeding 1 and 50 μM, respectively, were toxic to the cells and 3-methylcholanthrene was found to be cytotoxic at all concentrations tested (0.01–1 μM).

In order to observe any increases in the specific protein content of the three enzymes, we utilized Western immunoblot analysis of whole-cell homogenates. As seen by Mathis *et al.* (9), inclusion of 1 μM DEX in the culture medium potentiates induction of P450IA1 protein by 100 μM BA by 1.5-fold, as measured by densitometric scanning (Fig. 6, Table 2). The changes in GST subunit content of fetal cell cultures that were treated with 100 μM BA plus 1 μM DEX for 72 hr indicated that the Ya subunits were selectively potentiated approximately 28-fold, compared with cells treated with BA alone, which resulted in a 16-fold increase (Fig. 6); the potentiation seen in four Western immunoblot analyses ranged from 1.5- to 2.5-fold. The Yc subunit was potentiated even more, as seen in Fig. 6. In contrast, the Yb subunit did not appear to be induced by either BA or BA plus DEX. The immunoreactive band observed below the Yb subunit may represent the Ya subunit or another immunoreactive species. Although QR enzyme activity of fetal cell cultures was not increased by DEX (Table 1), the induction of protein content by 100 μM BA was apparently potentiated 80-fold in the presence of 1 μM DEX (Fig. 6). DEX in the presence of BA caused a further 2.5-fold increase in the content of the QR. The increased protein content of QR in the presence of BA plus DEX is in striking contrast to the activity measurements presented in a previous section (Fig. 1, Table 1).

Induction of GST by glucocorticoids alone in cultured fetal rat hepatocytes. In the absence of PAH, concentrations of DEX exceeding 1 μM increased GST activity 2- to 3-fold (100 μM, 72 hr) in cultured fetal rat hepatocytes, compared with solvent controls (Figs. 3 and 4). QR activity was also increased by addition of concentrations of DEX found to increase GST activity in culture (control, 16 ± 6 nmol/min/mg; 100 μM DEX, 40 ± 5 nmol/min/mg). However, the concentration-response profiles for DEX alone (Fig. 3) and the temporal profiles (Fig. 7) for the increase in cellular GST activity in response to DEX did not parallel that seen for the increase in cellular tyrosine aminotransferase activity (Fig. 3), indicating that GST induction by high concentrations of DEX may not be mediated by a classical glucocorticoid-mediated event. However, the glucocorticoids DEX, CC, and hydrocortisone all increased GST activity 2- to 3-fold, compared with solvent controls (Fig. 4). Deoxycorticosterone, PCN, and other classes of steroid hormone examined failed to induce GST activity.

Western immunoblot analysis of the content of specific GST subunits of cultured fetal hepatocytes treated with DEX (100 μM, 72 hr) indicated that the Ya/Yc subunits and the Yb subunit were all increased approximately 9-fold, compared with solvent controls (Fig. 8, Table 2). QR protein content was also elevated 58-fold in response to high concentrations of DEX (Fig. 8). The lower immunoreactive band, observed on pro-

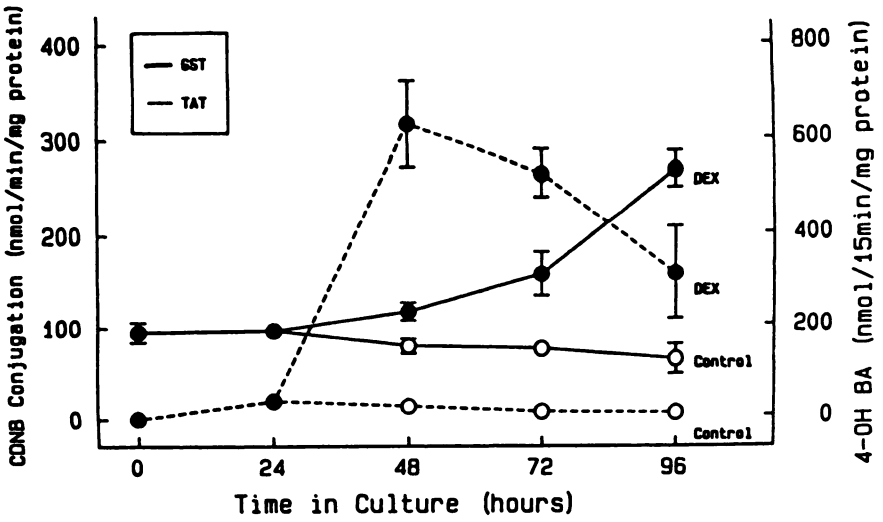

Fig. 7. Time course of induction of GST and tyrosine aminotransferase activities by DEX in cultured fetal rat hepatocytes. Cells were initially cultured in the absence of DEX. After 24 hr in culture, the medium was replaced and the cells were maintained for 72 hr in the absence (○) or presence (●) of DEX (100 μM). ——, GST activity; - - -, tyrosine aminotransferase. Each *point* represents the mean ± standard error of data obtained from four replicate dishes.

longed exposure, is presumed to be a proteolysis product, which also was observed at low levels in the purified enzyme preparation. High concentrations of DEX had no effect on either P450IA1 activity or protein content; addition of DEX to hepatocytes in culture does result in increased levels of cytochrome IIIA1 (P450p) as shown by Schuetz *et al.* (1, 2) with adult rat hepatocytes and with fetal rat hepatocytes in culture (data not shown).

Discussion

Our studies to identify other enzyme systems whose induction by PAH is synergized by glucocorticoids have indicated that at least one subunit of the GSTs (Ya) is synergistically increased by DEX (1 μM) in the presence of the primary inducing agent BA in cultured fetal liver cells. This result leads to a 2-fold increase in GST activity, similar to the changes previously observed with P450IA1 in cultured hepatocytes (9) and in intact animal (10). Additionally, higher concentrations of glucocorticoids (100 μM) induce GST activity and specific protein content in the absence of PAH. The GSTs composed of dimers of Ya, Yb, and Yc subunits are inducible by a diverse array of xenobiotics including PAH (28). mRNAs for each transferase subunit are thought to be regulated independently, although the Ya subunit, and to a lesser extent the Yb and Yc subunits, are coordinately elevated by 3-methylcholanthrene (28, 29). The 5′-flanking region of the rat liver GST Ya gene contains a PAH regulatory sequence for inducible expression by 3-methylcholanthrene (30). Consensus sequences for other regulatory elements in the rat gene, such as a glucocorticoid regulatory region, have yet to be defined.

Administration of pharmacological doses of glucocorticoids to neonatal rats has been shown to elicit precocious development of hepatic GST (15). DEX has been shown to increase P450IIIA1 levels by transcriptional activation but increased P450IIB1 levels by mRNA stabilization (3). In contrast, levels of P450IA1 are not altered by DEX. In our studies, the presence of DEX alone at much higher concentrations in cell culture (100 μM) also caused a 2- to 3-fold increase in GST activity and a 9-fold increase in Ya, Yb, and Yc subunit content. This response is similar to that seen during induction of cytochrome P450IIIA1 (2), NAD(P)H-cytochrome P450 reductase (3), γ-glutamyl transpeptidase (6), and tyrosine aminotransferase (31) by pharmacological doses of DEX.

Detailed studies of the tyrosine aminotransferase gene have indicated the presence of multiple glucocorticoid-responsive elements, which are located upstream from the initiation site of transcription (31). A proximal site does not function by itself but, in the presence of a distal responsive element, the proximal site confers a synergistic increase in gene expression of tyrosine aminotransferase by glucocorticoids. The consensus hexanucleotide sequence [TGT(C/T)CT] implicated in glucocorticoid receptor binding (31, 32) is also present near the junction between exon I and intron I of the mouse GST gene coding for the Ya subunit (33). In addition to glucocorticoids, which appear to positively regulate the expression of the GSTs, thyroid hormone has been shown to negatively regulate the levels of GST Ya/Ya homodimer in rat liver (34). These data would suggest that the regulation of this enzyme family is under multihormonal control.

QR activity and protein content were also induced by BA in cell culture. Interestingly, concentrations of DEX that were without effect on QR activity apparently increased the amount of immunoreactive protein in cells treated with BA (Fig. 6). Additionally, treatment of cells with a higher concentration of DEX alone resulted in the detection of increased levels of QR-immunoreactive proteins. The presence of at least two isozymes of QR in rat liver has been described (35). Our immunoelectrophoretic data would suggest that low concentrations of DEX may selectively potentiate BA induction of at least one form of the QR subunit. Treatment of cells with higher concentrations of DEX alone may also induce a form of the QR that exhibits little or no enzyme activity toward NADPH and menadione-dependent cytochrome *c* reduction. The presence of glucocorticoid-responsive elements in the QR gene have yet to be defined. Interestingly, the regulation of expression of QR by certain chemical carcinogens has been suggested to be dependent on increased transcriptional activity related to the methylation status of the QR gene. This decrease in methylation status apparently results in a marked elevation of the mRNA content specific for QR. This appears to be the case for QR found in persistent hyperplastic nodules caused by chemical carcinogens in rat liver (36).

Detailed studies of the tyrosine aminotransferase gene have

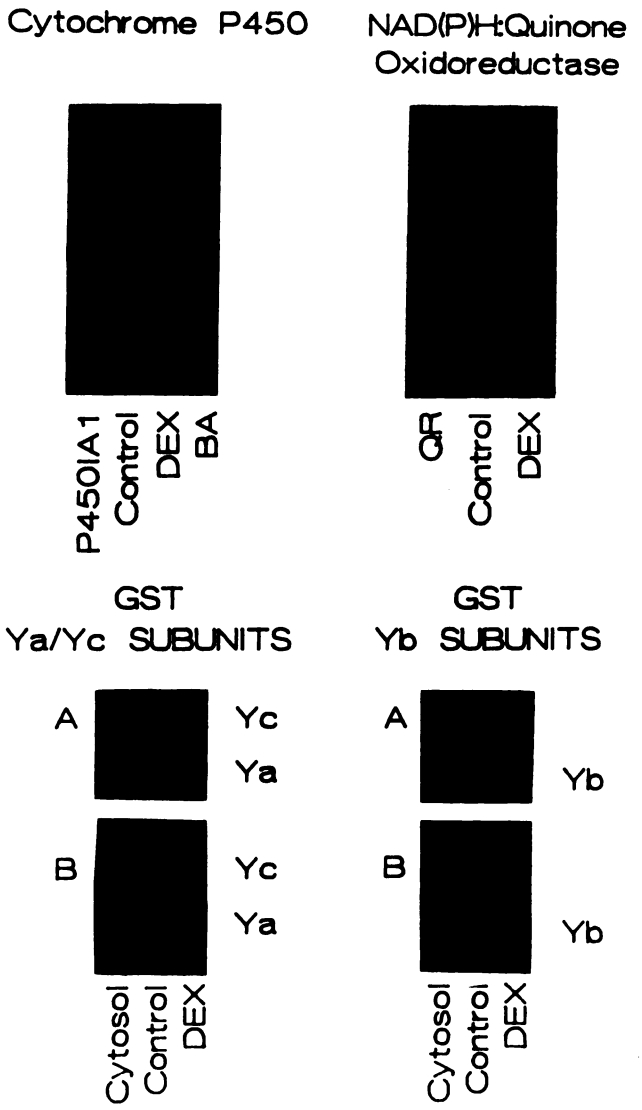

Fig. 8. Western immunoblot analyses of P450IA1, QR, and GST Ya, Yb, and Yc subunit content of fetal rat hepatocytes maintained in culture in the presence of high concentrations of DEX. The *first lane* of each *panel* is a standard of either pure protein (P450IA1, 25 ng; QR, 300 ng) or liver cytosol (cytosol, 5 μg of protein) obtained from male Sprague-Dawley rats treated with 3-methylcholanthrene. Cell protein (10 μg) was from fetal rat hepatocytes maintained in culture for 72 hr in the absence (*control*) or presence of 100 μM DEX (*DEX*). Two exposure times were used for the Western blots using anti-GST Ya/Yc and Yb globulin to demonstrate the induction of the transferase subunits. A, 6-hr exposure of X-ray film; B, 18-hr exposure of X-ray film.

indicated a functional role for the presence of multiple glucocorticoid response elements located upstream from the initiation site (31). A site proximal to the coding region of the tyrosine aminotransferase gene apparently does not function by itself, but in the presence of a distal GRE, the proximal site confers a synergistic increase of gene expression of tyrosine aminotransferase by glucocorticoids. This regulatory mechanism involving the function of two sets of response elements on DNA is analogous to our observations with P450IA1 (12) and possibly to our current studies on GST. The *Ah* receptor is obligatory for initiation of gene expression of P450IA1 (37, 38) and GST (30). Whereas low concentrations of glucocorticoids by themselves have no effect on the expression of these enzymes in the absence of PAH, the occupancy of the glucocorticoid receptor must synergize PAH induction by binding at a site on intron 1 of P450IA1 (11, 12). Similar inductive responses have been reported for γ-glutamyl transpeptidase (6). We propose that a glucocorticoid-dependent mechanism for gene expression may also hold for the Ya subunit of GST and for QR.

In addition, induction of GSTs and QR activity/protein content at high concentrations of glucocorticoids suggests that these steroid derivatives may also increase expression of these proteins by a nonclassical mechanism, in a manner similar to that shown by Schuetz and Guzelian for P450IIIA1 (2). Collectively, these data are indicative of the importance of glucocorticoids in the regulation of hepatic xenobiotic metabolism throughout development.

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