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Regulation of Cytochrome P-450b/e and P-450p Gene Expression by Growth Hormone in Adult Rat Hepatocytes Cultured on a Reconstituted Basement Membrane*

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Growth hormone (GH) may directly affect the expression of some liver cytochromes P-450 inducible by xenobiotics, but this has been difficult to establish with pituitary ablation in living animals. Therefore, we incubated adult rat hepatocytes on a laminin-rich matrix, matrigel, a new system that permits hepatocytes to respond to xenobiotics with induction of the microsomal hemoproteins, the cytochromes P-450, in culture as they do in the intact liver (Schuetz, E. G., Li, D., Omiecinski, C. J., Muller-Eberhard, U., Kleinman, H. K., Elswick, B., and Guzelian, P. S. (1988) *J. Cell. Physiol.* 134, 309–323). Indeed, when cultures were treated with phenobarbital, there was a rise in mRNAs for the cytochromes P-450b/e and P-450p accompanied by a rise in mRNA for 5-aminolevulinate synthase, the rate-limiting enzyme in heme biosynthesis. Analysis of nuclei from the matrigel cultures established that phenobarbital treatment had activated transcription of the P-450b/e gene. Co-incubation of the cultures with physiologic concentrations of growth hormone completely blocked the induction of these P-450 mRNAs and partially blocked the rise in 5-aminolevulinate synthase mRNA. Induction of P-450p by isosafrole also was inhibited strongly by GH, whereas P-450p induction by pregnenolone 16α-carbonitrile or dexamethasone was affected only weakly by GH. The effect of GH was specific inasmuch as phenobarbital-inducible expression of P-450 reductase, glucocorticoid-inducible expression of tyrosine aminotransferase, and basal expression of albumin were unaffected by the presence or absence of growth hormone. Nuclear analysis revealed that growth hormone inhibited phenobarbital-induced P-450b/e gene transcription, whereas the hormone was without effect on transcription of the liver-specific gene, tyrosine aminotransferase. In contrast, the addition of another peptide hormone, insulin-like growth factor I, was without inhibitory effect on P-450 gene expression. We conclude that growth hormone acts specifically and selectively in direct contact with the hepatocyte to control xenobiotic induction of some liver drug-metabolizing enzymes.

Mammalian hepatocytes contain a congeries of microsomal hemoproteins, collectively termed the cytochromes P-450, which catalyze the oxidation of numerous foreign compounds (xenobiotics) such as drugs and environmental pollutants as well as many endogenous substrates. Expression of these cytochromes is governed by a supergene family that appears to be under multifactoral regulatory control. As a classic example, the liver responds to administration of such lipophilic drugs as phenobarbital (2) with increased *de novo* synthesis of heme and apocytochromes for P-450b/e (3) and P-450p (4), representatives of the class II and class III cytochrome P-450 gene families, respectively (1). The selective increase in the products of these cytochrome genes, often termed microsomal enzyme induction, is believed to be an important physiologic mechanism whereby the hepatocyte can adapt to changes in its exogenous chemical environment. However, because the levels of the cytochromes P-450 may be increased or decreased in association with age, gender, diet, and stress, among many other factors, it seems likely that endogenous substances can be inducers (or repressors) of these hemoproteins. Indeed, several years ago we showed that P-450p is inducible not only by phenobarbital but also by corticosterone, the major circulating glucocorticoid in the rat (5). Moreover, appropriate infusions of growth hormone (GH)[1] to hypophysectomized male rats elicits expression of P-450i, a cytochrome normally found only in the liver of adult female rats (6).

It is difficult to define the endogenous factors that regulate phenobarbital-inducible cytochrome P-450 genes in the living animal. Although cell culture would, in theory, be of great use for this purpose, most of the hepatocyte culture systems described to date express only small amounts (if any) of P-450b/e. Recently, we described a new system for primary monolayer culture of adult rat hepatocytes incubated on a substratum of matrigel, a reconstituted basement membrane prepared from extracts of the Engelbreth-Holm-Swarm sarcoma (7). Unlike standard cultures of hepatocytes attached to collagen (8), cultures on matrigel appear to have extended longevity and express numerous differentiated functions characteristic of the adult liver, including phenobarbital-responsive increases in P-450b/e mRNA and protein (7). We have

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[1] The abbreviations used are: GH, growth hormone; IGF-I and IGF-II, insulin-like growth factors I and II; PCN, pregnenolone 16α-carbonitrile; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid. Under the recommended P-450 gene nomenclature system (1), IIB1 and IIB2 refer to the genes previously designated P-450b/e, collectively referring to the major phenobarbital-inducible cytochromes P-450 in rat liver. IIIA1, previously designated P-450p, refers to the major glucocorticoid-inducible cytochrome P-450 in rat liver; whereas P-450j, the major ethanol-inducible P-450, is designated IIE1.

also discovered that use of matrigel permits the cultured hepatocyte to respond to some hormonal influences with appropriate alterations of cytochrome P-450 gene expression. For example, when cultures of hepatocytes isolated from a male rat are incubated on matrigel and constantly exposed to culture medium containing human GH, there is a reversal in the sex-dependent phenotype of cytochrome P-450 expression manifested by the induction of "female-specific" P-450i mRNA and protein (9).

Having such a remarkably responsive culture system available (7, 9) should make it possible to define rigorously the interactions between hormones and xenobiotics on control of liver gene expression. The present report will show that phenobarbital treatment of cultured hepatocytes results in a transcriptional activation of the P-450b/e gene accompanied by increased expression of the gene for aminolevulinate synthase, the rate-controlling enzyme in the heme biosynthetic pathway. We also show, for the first time, that this coordinated inductive response is under stringent inhibitory control by GH.

MATERIALS AND METHODS

Adult male Sprague-Dawley rats (Dominion Labs, Dublin, VA) weighing 180–200 g were maintained in wire bottom cages with free access to animal chow and water for 2 weeks prior to use. Collagenase type I was purchased from Cooper Biochemical Co., Malvern, PA. Human GH (somatotropin) (catalog no. S-4255) extracted from human pituitaries and prolactin extracted from sheep pituitary glands were purchased from Sigma. Prolactin was dissolved in Sigma hormone solvent. Recombinant methionyl human GH (lot GH007DAX) was a gift from Genentech Corp. (Palo Alto, CA). Recombinant insulin-like growth factor I (IGF-I) was a gift from Dr. Thomas Jeatran, Lilly. PCN was a gift from John Babcock (The Upjohn Co.).

Recombinant Plasmids—The cloned cDNAs that we used were gifts from the following investigators: rat P-450e (pR17), from Dr. Milton Adesnick (New York University Medical Center, New York) (10); tyrosine aminotransferase (pTAT), from Dr. Gunter Schutz (German Cancer Research Center, Heidelberg, Federal Republic of Germany) (11); albumin from Dr. Lola Reid (Albert Einstein Medical Center, Bronx, NY) (12); P-450 reductase (pOR-7), from Dr. Charles Kasper (McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI) (13); rat P-450p (a full-length cDNA corresponding to PCN-1 (14)) and ($pDex_{12}$) (15), human P-450j (16), and a rat 5-aminolevulinate synthase cDNA have been prepared previously by our laboratories (17).

Preparation of Hepatocyte Cultures on Matrigel—To summarize, matrigel was prepared from Engelbreth-Holm-Swarm sarcoma propagated in C57BL/6J female mice and was stored at −20 °C (7). Shortly prior to its use, matrigel was warmed to 4 °C and was applied (1 mg/100 μl) evenly to the bottom of 60-mm plastic dishes. Gelation was complete within 30–60 min at room temperature. As described in detail (8), freshly isolated hepatocytes (prepared by nonrecirculating perfusion of collagenase through the portal vein of ether-anesthetized rats) were inoculated (3.5×10^6/dish) in 3 ml of our standard serum-free medium. This medium is a modification of Waymouth 752, containing amino acids, salts, vitamins, and minerals (zinc, selenium), with insulin (1.5×10^{-7} M) as the only hormone (8). Cultures were maintained in a humidified incubator at 35 °C in an atmosphere of 5% CO_2, 95% air. The medium was renewed daily, with steroids and other inducers added as described previously (7).

Northern Blot Analyses—Total RNA (10 μg) isolated from the pooled cells from three to five culture dishes (7) was resolved by electrophoresis on denaturing formaldehyde-agarose gels and was blotted by capillary action onto nitrocellulose (4). Nitrocellulose sheets were prehybridized, hybridized with a radioactively labeled probe containing ^{32}P, and then washed. The bands were visualized with the use of autoradiography (4). Evidence that mRNA was equally loaded and transferred was obtained by equivalent intensity of ethidium bromide staining of 18 and 28 S ribosomal RNA bands.

Nuclei Isolation—Nuclei were isolated from rat hepatocytes by a modification of published procedures (18). Briefly, cells from 16 dishes were scraped in media, pelleted at 1000 × *g*; washed once with phosphate-buffered saline; resuspended in modified reticulocyte standard buffer—a hypotonic lysing buffer consisting of NaCl (2 mM), Tris-HCl (10 mM), pH 7.4, $MgCl_2$ (35 mM), 2-mercaptoethanol (14 mM), $CaCl_2$ (2 mM), Hepes, pH 7.4 (20 mM), spermidine (0.5 mM), and EDTA, pH 7.5 (0.1 mM)—and incubated at 4 °C for 5 min. After addition of Triton X-100 (to 0.2%), the cells were lysed (90–100%) in a Dounce homogenizer with tight fitting pestle. KCl (to 0.03 M) was added, and the samples were spun at 1,000 × *g* to sediment the nuclei. The nuclei were suspended in 12 ml of modified buffer consisting of Tris-HCl, pH 8.1 (10 mM), glycerol (20%), KCl (140 mM), $MgCl_2$ (5 mM), $MnCl_2$ (1 mM), 2-mercaptoethanol (14 mM), and spermidine (0.5 mM), and the suspension was filtered through gauze. The nuclei were sedimented at 1,000 × *g* for 5 min, washed once with buffer, and stored for no more than 1 week at −80 °C.

Transcription in Isolated Nuclei and Hybridization—Transcription in isolated nuclei, isolation of [^{32}P]RNA, and hybridization to nitrocellulose paper were performed as described previously (19). DNA content of the nuclei was measured fluorometrically with the use of Hoescht dye 33258. Assuming 1 pg of DNA/nucleus, $2–6 \times 10^7$ nuclei were used in the transcription reaction. Briefly, equal amounts of [^{32}P]RNA (constant cpm/μl of hybridization solution) were hybridized to cDNA inserts (50 ng) immobilized on nitrocellulose filters. Filter hybridization was carried out at 42 °C for 96 h in a final volume of 0.4 ml containing 1×10^6 cpm. The nitrocellulose strips were washed, and the amounts of [^{32}P]RNA transcripts were quantitated by autoradiography. The amounts of hybridization were determined to be similar by measuring the efficiency of hybridization with an antisense [^3H]cRNA to tyrosine aminotransferase prepared (by subcloning the 1.1-kilobase rat tyrosine aminotransferase cDNA into the *Eco*RI site of the vector pGEM 3(Z) followed by RNA synthesis utilizing [^3H]UTP as the radiolabel as described by the manufacturer (Promega Biotec, Madison, WI; Technical Bulletin 002)) and included in each hybridization mixture (1000 cpm/assay).

RESULTS

In preliminary experiments, we found that hepatocytes prepared from a male rat and incubated for 5 days on matrigel in our standard culture medium resembled the parent tissue in containing readily detectable amounts of mRNA for P-450p, a cytochrome expressed in male but not female liver (20), but little (if any) mRNA for P-450b/e, a pair of cytochromes present only in very small amounts in either sex in adult rats[2] (21). These results are consistent with our previous report of finding immunoreactive P-450p protein but not P-450b/e protein in hepatocyte cultures (22). We also confirmed that cultures on matrigel respond to additions of foreign compounds to the medium with induction of cytochromes P-450, as has been established for the liver *in vivo*. For example, P-450p mRNA was induced strongly by a synthetic steroid, pregnenolone 16α-carbonitrile (PCN), and moderately by phenobarbital or isosafrole (Fig. 1). Also in culture, as *in vivo* (20), phenobarbital and isosafrole were strong inducers of P-450b/e mRNA, whereas PCN gave only a weak response (Fig. 1). However, when some of these cultures from the same liver were incubated in medium supplemented with human GH, induction of P-450p mRNA and P-450b/e mRNA by all three xenobiotic inducers was suppressed (Fig. 1). Additions to the media of 10 milliunits/ml (8.8 μg/ml) recombinant human methionyl GH also suppressed induction of P-450b/e and P-450p mRNA by PB.[2] This inhibitory effect was specific for the somatogenic properties of GH inasmuch as additions of equivalent amounts of another pituitary hormone, prolactin, were without effect on inducible expression of mRNAs for P-450b/e or P-450p (Fig. 1). To compare the inhibitory effects of GH on cytochromes P-450, we examined the mRNA for P-450 reductase, a companion microsomal enzyme that is induced by PCN (4.9-fold), phenobarbital (6.7-fold), and isosafrole (6.8-fold) in hepatocytes cultured on matrigel.[2] P-450 reductase mRNA induction in culture was unaffected by GH

[2] E. G. Schuetz, J. D. Schuetz, B. May, and P. S. Guzelian, unpublished experiments, not shown.

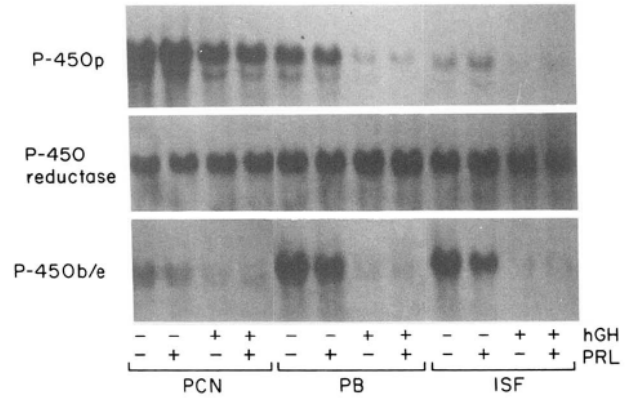

FIG. 1. **Effect of growth factors on the inducible expression of cytochrome P-450 mRNAs in cultures of rat hepatocytes maintained on matrigel.** Freshly isolated hepatocytes prepared as described under "Materials and Methods" from an untreated male rat were inoculated on dishes precoated with matrigel and were incubated from the time of plating (day 0) with or without human GH (*hGH*) at 10 milliunits/ml and prolactin (*PRL*, 20 milliunits/ml). PCN (10 μM), phenobarbital (*PB*, 2 mM), or isosafrole (*ISF*) (15 μg/ml) was incorporated into the culture medium beginning on day 3. RNA harvested from the cells on day 5 was analyzed on Northern blots and hybridized with either a cDNA probe for P-450b/e, P-450p, or P-450 reductase.

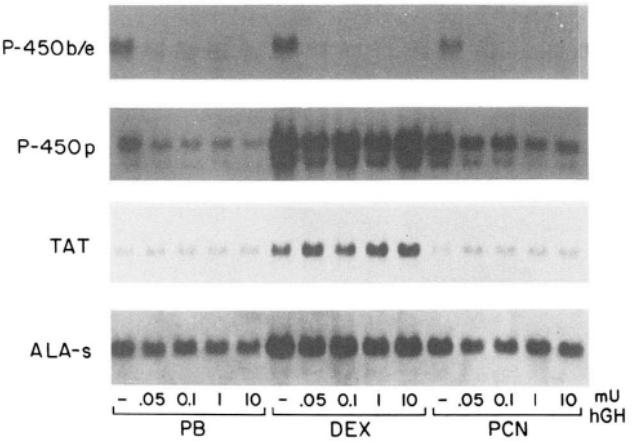

FIG. 2. **Effect of growth hormone concentrations on gene expression in cultures of rat hepatocytes maintained on matrigel.** Hepatocytes (prepared as described in Fig. 1) were maintained in Waymouth 752 medium containing different amounts of human GH (0.05–10 milliunits/ml). Phenobarbital (*PB*, 2 mM), dexamethasone (*Dex*, 10 μM), or PCN (10 μM) was added to the medium beginning on day 3. Cells were harvested on day 5. RNA was extracted and was analyzed on Northern blots hybridized sequentially (see "Materials and Methods") with cDNA probes specific for P-450b/e, P-450p, tyrosine aminotransferase (*TAT*), or 5-aminolevulinate synthase (*ALA-s*).

(Fig. 1). This result is consistent with results reported for living rats (23).

The liver is a major site for degradation of GH (24). Therefore, we used a supraphysiologic concentration (10 milliunits/ml = 8.8 μg/ml) of GH in the initial experiments (Figs. 1 and 2) to ensure continual exposure of the hepatocytes to GH. In addition, this protocol would likely mimic the nonpulsatile pattern of GH secretion in female rats (25). Nevertheless, exposure of male hepatocytes to phenobarbital plus a lower concentration of GH (0.05 milliunits/ml = 44 ng/ml) within the physiologic range of 20–500 ng/ml in rat serum (26) still suppressed the induction of P-450p mRNA (75–80%) and the induction of P-450b/e mRNA almost completely by phenobarbital (Fig. 2). This low concentration of GH also blocked the induction of P-450b/e mRNA by dexamethasone or by PCN (Fig. 2). In contrast, the induction of P-450p mRNA by the non-phenobarbital compounds, dexamethasone and PCN, was only partly suppressed (44 and 37%, respectively) by GH (Figs. 1 and 2). It is also unlikely that these results represent either nonspecific toxicity or inhibited uptake of the inducers because on some of the same Northern blots of RNA extracted from dexamethasone-treated cultures, induction of tyrosine aminotransferase mRNA was unaffected by the presence of GH (Fig. 2). Similarly, none of the GH doses we tested altered the amounts of mRNA for albumin or for tyrosine aminotransferase in cultures maintained on matrigel in the absence of a P-450 inducer.[2]

If matrigel allows induction of some cytochromes P-450 to occur in cultured hepatocytes as *in vivo*, then there should be a corresponding increase in heme formation, measurable as a rise in 5-aminolevulinate synthase, the rate-limiting enzyme in the heme biosynthetic pathway. Indeed, when we treated hepatocyte cultures with phenobarbital, with dexamethasone, or with PCN at the doses used in the preceding experiments (Figs. 1 and 2), the levels of 5-aminolevulinate synthase mRNA hybridizable with a rat liver 5-aminolevulinate synthase cDNA on Northern blots rose by 2–5-fold.[2] Since heme availability may limit formation of P-450 mRNAs (27), GH could inhibit formation of cytochromes P-450 by inhibiting heme synthesis. However, addition of heme to the cells in a form (heme/albumin, 100 μM final) that is metabolically active (28) failed to reverse the suppression of phenobarbital induction of P-450b/e mRNA by GH.[2] Furthermore, GH actually decreased 5-aminolevulinate synthase mRNA in control cultures (60%) and induction in cultures treated with phenobarbital (42%), dexamethasone (63%), or PCN (58%) (Fig. 2). Since blocked heme synthesis would be expected to increase 5-aminolevulinate synthase mRNA (29), it is unlikely that GH suppresses P-450b/e induction by creating a deficiency in cellular heme. These combined results demonstrate that the suppression of inducible P-450b/e and P-450p expression by GH is selective for both the mRNA examined and for the inducer employed.

Because GH produces some of its effects secondarily by stimulating the liver to produce somatogenic peptides (somatomedins (30, 31)), we incubated 48-h-old hepatocytes in the presence or absence of either GH or of IGF-I for 3 days and added inducers for the last 48 h. We found, once again, that GH suppressed induction of P-450b/e mRNA and P-450p mRNA by PCN, by phenobarbital, or by isosafrole, and yet high concentrations (200 ng/ml) of IGF-I failed to suppress these responses.[2]

GH could exert its effects on induction of P-450b/e and P-450p mRNAs by inhibiting gene transcription or by altering mRNA stability. To estimate the rate of gene transcription in the cultured hepatocytes, nuclei isolated from the cells were incubated with radioactive RNA precursors, and "run-off" transcripts were isolated for analysis. In three separate experiments, assay of nuclei from cultured hepatocytes treated with phenobarbital for 3.5–6 h (when the rate of P-450b/e transcription should be maximal in rat liver (32)) showed an increase of 3–4-fold over control values (Fig. 3). In contrast, analysis of nuclei from cultures incubated in medium containing GH prior to and concurrent with phenobarbital treatment showed that the induced P-450b/e transcription had been suppressed completely (Fig. 3). Likewise, Northern blot analysis of nuclear RNA isolated from phenobarbital-treated cultures and probed with pR17 detected increased expression of a phenobarbital-inducible transcript that was suppressed by concurrent GH treatment. There was a low, basal rate of P-

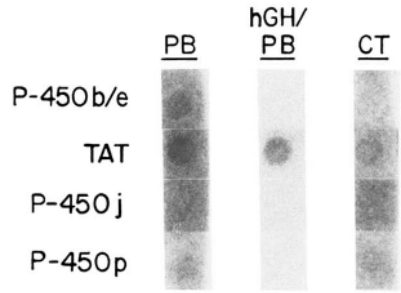

FIG. 3. **Effect of growth hormone on transcription of liver gene in hepatocytes cultured on matrigel.** Hepatocytes from a male rat were incubated (for 120 h) in medium containing GH (0.05 milliunits/ml). The cultured hepatocytes were then transferred to media supplemented with "soluble" matrigel (1 ml of gel/100 ml of media) plus phenobarbital (*PB*, 2×10^{-3} M) in the presence or absence of GH or control (*CT*) medium. After 3.5 h, nuclei were harvested and then incubated with [^{32}P]UTP. The radiolabeled nuclear RNA was isolated and hybridized to dot blots of immobilized cDNAs (see "Materials and Methods"). *TAT*, tyrosine aminotransferase.

450p transcription detectable in untreated control cultures (Fig. 3), a finding consistent with the presence of detectable amounts of P-450p mRNA at 5 days in culture (not shown). However, continual treatment of cultures with media containing GH (mimicking the female pattern) suppressed the basal level of P-450p transcription in these cells (Fig. 3), consistent with the general suppressive influence of GH on expression of P-450p (33). Moreover, this result is consistent with the disappearance of basal P-450p mRNA in GH-treated cultures.[2] Nevertheless, GH was by no means a nonspecific inhibitor of hepatocyte gene transcription. The peptide had no effect on the spontaneous rate of tyrosine aminotransferase gene transcription (Fig. 3). Likewise, there was no effect of phenobarbital or GH on the rate of transcription of P-450j (Fig. 3). From these experiments, we conclude that regulation of the phenobarbital-inducible P-450b/e gene expression in hepatocytes cultured on matrigel prominently involves hormonally sensitive events controlling gene transcription.

DISCUSSION

The present studies disclose the mechanism of induction of P-450b/e by xenobiotics and document that this process is under direct inhibitory control by GH. These experiments would not have been possible without use of primary hepatocyte cultures maintained on a basement membrane matrix, matrigel. In the conventional system of hepatocytes cultured on type I collagen (Vitrogen), induction of P-450b/e mRNA by phenobarbital was undetectable (7), whereas induction of P-450b/e protein synthesis requires a sensitive radiometric immunoprecipitation assay for its detection (34). However, when cultured on matrigel, the hepatocyte responds to xenobiotic inducers with enhanced formation of proteins and mRNAs for P-450b/e and for P-450p accompanied by a rise in 5-aminolevulinate synthase mRNA (Fig. 2), as is seen *in vivo* (7, 15). Most important, we have demonstrated for the first time that phenobarbital stimulated transcription of P-450b/e genes in culture as reported for the intact liver (10, 35). Indeed, in other primary hepatocyte culture systems, transcription of many liver-specific genes ceases even though decreased mRNA degradation may actually "induce" mRNA content, dramatically (12).

In contrast to Vitrogen, matrigel appropriately permits expression of many liver-specific functions in culture. This effect may be linked to the rounded rather than flattened shape that the hepatocytes assume when cultured on matrigel (7, 36, 37). Another possibility is that matrigel contains, in addition to laminin and type IV collagen, heparan sulfate proteoglycans, which restore transcription of some liver-specific mRNAs in primary hepatocyte cultures (38). Since the present data show that matrigel is critical also for responsiveness of the cytochromes P-450 to the presence of GH in the medium, the newly recognized importance of xenobiotic interactions with hormones in the liver can be better defined with the matrigel culture system.

Our results in culture document that GH exerts a potent and selective inhibitory effect on P450b/e. The concept of GH as an inhibitor of liver gene expression contrasts with the general idea that GH is hepatotrophic, stimulating such functions as glutamine synthetase (39) and $\alpha_{2\mu}$-globulin (40). Indeed, it has been proposed that GH is responsible for the sexual dimorphism in expression of some liver cytochromes P-450 in rats (41). Injections of GH to hypophysectomized rats simulate the normal male pulsatile pattern of GH release and induce male-specific P-450h expression in the liver, whereas continuous infusions of GH mimic the normal female pattern of GH release and induce female-specific P-450i (42). It has also been claimed that GH decreases expression of P-450b/e (43) and P-450p (44) *in vivo* because these cytochromes increased following hypophysectomy of male rats and declined when those animals were given GH. However, others report that hypophysectomy has no effect on P-450b/e or P-450p (45). Our use of hepatocyte cultures circumvents the ambiguities involved in endocrine ablations of living animals and establishes unequivocally that GH acts on the liver directly to block phenobarbital induction of P-450b/e and P-450p. Also, our findings are consistent with the idea that intermittent or continuous exposure of rats to GH suppresses full expression of P-450p measured in whole animals as testosterone 6β-hydroxylase activity (6, 33) or as ethylmorphine *N*-demethylase activity (46). Indeed, from our tests of steroid inducers in culture (Fig. 2) we can exclude the suggested possibility that dexamethasone induces P-450b/e content in hypophysectomized rats indirectly by decreasing GH release from the pituitary (43). Similarly, it may be inappropriate to administer systemically acting agents like cycloheximide and conclude that inhibition of protein synthesis in the liver accounts for inhibition of phenobarbital-stimulated P-450b/e transcription unless effects on pituitary release of GH have been ruled out (47). Moreover, if GH is preferentially cleared by the periportal hepatocytes as is another peptide hormone, epidermal growth factor (48), then our data may explain the failure of phenobarbital to induce P450b/e in periportal hepatocytes as contrasted with the induction that does occur in pericentral hepatocytes (49).

Underscoring the striking selectivity of the GH effect is the observation that GH suppressed induction of P-450p mRNA in cultured hepatocytes by phenobarbital and yet had only a partial inhibitory effect on induction of P-450p by dexamethasone (Fig. 2). There may be several closely related forms of P-450p, each under qualitatively distinct regulation (50), or GH may act at different steps in the induction process of a P-450p gene induced in common by steroids and by phenobarbital. We were able to exclude heme deficiency, secondary to inhibition of 5-aminolevulinate synthase induction, as a possible mechanism by which GH affects P-450b/e or P-450p induction. Although GH also suppresses liver P-450j (29) in rats, this peptide is not a universal cytochrome P-450 repressor. GH appears to induce P-450h in male rat liver (51) and strongly increases expression of P-450i in hepatocytes cultured on matrigel (9).

Inhibition of phenobarbital induction of mRNAs for P-450b/e and P-450p in hepatocyte cultures was detected within

3 days of GH treatment, although maximum suppression required 5 days of incubation in medium containing the peptide. In fact, the least suppressive effect was observed when cells without GH pretreatment received GH plus phenobarbital[2] for 1 day, concurrently. The requirement of 5 days for this suppressive effect, reported also for induction of lipogenic enzymes by GH in hepatocyte cultures (52) and of P-450i in the liver of hypophysectomized rats (42), suggests that GH may act by fostering the accumulation of a second mediator such as IGF-I. Indeed, IGF-I treatment has been shown to increase $P\text{-}450_{AROM}$ mRNA in human ovarian granulosa cells (53), whereas IGF-II regulates the cholesterol side chain cleavage enzyme, $P\text{-}450_{scc}$ (54). However, addition of IGF-I to the hepatocyte cultures did not reproduce the effects of GH suppression of P-450b/e and P-450p. Furthermore, even though female rats, which maintain a higher continuous level of plasma GH, are not as responsive to induction by phenobarbital of P-450b/e as are males (43), induction of P-450b/e is not totally suppressed, as is seen here in hepatocytes maintained in medium supplemented with GH (Figs. 1 and 2). Therefore, it is likely that other factors in the intact animal modulate the inhibitory effect of GH on induction of P-450b/e. It should be possible to identify these postulated counteracting factors with experiments in the matrigel culture system.

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