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BIPHASIC REGULATION OF CYTOCHROME P450 2B1/2 mRNA EXPRESSION BY DEXAMETHASONE IN PRIMARY CULTURES OF ADULT RAT HEPATOCYTES MAINTAINED ON MATRIGEL

THOMAS A. KOCAREK,*† ERIN G. SCHUETZ‡§ and PHILIP S. GUZELIAN*‖

*Division of Clinical Toxicology and Environmental Medicine, Department of Medicine, and ‡Department of Pathology, Medical College of Virginia, Richmond, VA, U.S.A.

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Abstract——We have demonstrated recently that although rat hepatocytes rapidly lose their cytochrome P450 mRNA content following their introduction into primary culture, hepatocytes cultured on Matrigel, a reconstituted basement membrane, subsequently spontaneously "reexpress" the mRNAs of some constitutive P450 forms (Kocarek *et al.*, *Mol Pharmacol* **43**: 328–334, 1993). In the present study, we used the Matrigel cell culture system to examine the dose-dependent effects of dexamethasone (DEX) treatments on the mRNAs for two of the P450 forms that are reexpressed spontaneously between days 3 and 5 in culture, 2B1/2 and 2C6. Treatment of cultured hepatocytes with low doses of DEX (10^{-9} to 10^{-8} M) that induced the mRNA for tyrosine aminotransferase, a model glucocorticoid-inducible gene, suppressed the spontaneous appearance of 2B1/2 mRNA while having little or no effect on the level of 2C6 mRNA or on β-actin mRNA. However, treatment of the hepatocyte cultures with high doses of DEX (10^{-6} to 10^{-5} M) that induced P450 3A1 mRNA increased the amounts of the 2B1/2 and 2C6 mRNAs (4.1- and 2.4-fold, respectively, at 10^{-5} M DEX). In contrast to the suppressive effects on the spontaneous increases in 2B1/2 mRNA, low doses of DEX (10^{-8} to 10^{-7} M) enhanced the induction of 2B1/2 mRNA by phenobarbital (2.5-fold at 10^{-7} M DEX). Treatment of the hepatocyte cultures with triamcinolone acetonide, another potent glucocorticoid, suppressed spontaneous 2B1/2 mRNA expression at low doses, but did not induce 2B1/2 mRNA at high doses. Treatments with steroids of other classes, including dihydrotestosterone, 17α-ethinylestradiol, fludrocortisone or R-5020, failed to suppress 2B1/2 mRNA levels at low doses. Additionally, treatment with RU-486, a glucocorticoid/progestin receptor antagonist, induced 2B1/2 mRNA at high doses (10^{-6} to 10^{-5} M). The suppressive effects of DEX on spontaneous 2B1/2 mRNA expression observed at low doses are consistent with a classical glucocorticoid-mediated mechanism, while the high-dose inductive effects of DEX appear to be exerted through a nonclassical mechanism, perhaps akin to that for induction of 3A1.

Key words: cytochrome P450; CYP450; glucocorticoid; dexamethasone; hepatocyte; gene expression

The cytochromes P450 constitute a superfamily of hemoprotein monooxygenases, found prominently in liver, that function critically in the metabolism of lipophilic substances, both endogenous and xenobiotic. Some forms of hepatic P450 that are readily detected in the livers of untreated animals are also inducible by treatments with xenobiotics such as polycyclic aromatic hydrocarbons (1A2 and 2A1), PB¶ (2B2, 2C6, 2C7 and 3A2), ethanol (2E1) or peroxisome proliferators (4A1) [for review see Ref. 1]. A variety of experiments in living animals suggests that endogenous factors, including hormones, are responsible for maintaining the amounts of these "constitutive" forms in the basal steady state, but these have been difficult to define.

To investigate endogenous factors that regulate liver P450s, we have used primary cultures of rat hepatocytes maintained on plastic dishes coated with Matrigel, a reconstituted basement membrane extracted from the Engelbreth–Holm–Swarm sarcoma [2]. These hepatocyte cultures respond to treatments with P450 inducers such as PB with effects, including induction of 2B1/2, strikingly similar to those seen in treated rats [2–4]. The cultured hepatocytes also respond to metabolic factors including hormones. For example, exposing male rat hepatocyte cultures to growth hormone continuously, so as to mimic the female growth hormone secretion pattern, produces the appearance of the female-specific constitutive cytochrome 2C12 [5]. As another example of hormonal regulation, glucocorticoids induce liver P450 3A1 in rats [6] and in rat hepatocyte cultures [7].

In the course of examining the effects of P450 inducers, we found that the mRNAs for several constitutive liver P450s, including 2A1, 2B2, 2C6,

† Corresponding author: Thomas A. Kocarek, Ph.D., Institute of Chemical Toxicology, 2727 Second Ave., Room 4000, Detroit, MI 48201, U.S.A. Tel. (313) 963-7487; FAX (313) 577-0082.

§ Present address: Department of Pharmaceutical Sciences, St. Jude Children's Research Hospital, Memphis, TN, U.S.A.

‖ Present address: University of Colorado Health Sciences Center, 4200 E. 9th Ave., 723 BRB, Box B146, Denver, CO, U.S.A.

¶ Abbreviations: DEX, dexamethasone; EE, 17α-ethinylestradiol; GCR, glucocorticoid receptor; GRE, glucocorticoid responsive element; PB, phenobarbital; R-5020, promegestone; and TAT, tyrosine aminotransferase.

2C7 and 4A1, are present initially in freshly isolated hepatocytes and in cultures of these cells during the first few hours, disappear during the next 48 hr, and then reappear during days 2–5 [8]. Inasmuch as glucocorticoids affect expression of many P450s in the livers of rats [6, 9–11], we have critically examined the effects of the synthetic glucocorticoid, DEX on the amounts of the mRNAs for two constitutive P450 forms (2B2 and 2C6) that are strongly "reexpressed" in rat hepatocytes cultured on Matrigel. We report that DEX treatment produced opposite, dose-dependent effects on the amounts of 2B1/2 mRNA in the cultured hepatocytes in that low doses suppressed the spontaneous appearance of 2B1/2 mRNA, while high doses increased 2B1/2 mRNA levels.

MATERIALS AND METHODS

Materials. Adult male Sprague–Dawley rats (Dominion Laboratories, Dublin, VA) weighing 175–200 g were maintained in wire-bottomed cages with free access to animal chow and water for 2 weeks before use. Collagenase type I was purchased from the Cooper Biochemical Co. (Malvern, PA). Matrigel was prepared from the Engelbreth–Holm–Swarm sarcoma, as previously described [2]. PB was from J. T. Baker, Inc. (Phillipsburg, NJ) and DEX, triamcinolone acetonide, EE, dihydrotestosterone and fludrocortisone were from the Sigma Chemical Co. (St. Louis, MO). RU-486 and R-5020 were gifts from Roussel-Uclaf (Romainville, France). A cloned cDNA fragment to 3A1 (pDex12) [12] was isolated in this laboratory. A cDNA to P450 2B2 (pR17) [13] was supplied by Dr. Milton Adesnik (New York University, New York, NY), a cDNA to P450 2C6 [14] was provided by Dr. Frank Gonzalez (National Cancer Institute, Bethesda, MD), and a cDNA to TAT (pcTAT-2) [15] was furnished by Dr. Gunther Schutz (German Cancer Research Center, Heidelberg, Germany). A cDNA to rat β-actin was prepared by the polymerase chain reaction using amplimers purchased from Clontech (Palo Alto, CA).

Hepatocyte cultures and drug treatments. Rat hepatocytes were isolated from adult male Sprague–Dawley rats (230–280 g) and plated onto 60 mm plastic dishes coated with 150 μL Matrigel as previously described [2]. Cultures were maintained in a humidified incubator at 35° under an atmosphere of 5% CO_2/95% air. The culture medium, a modification of Waymouth MB-752 containing insulin (1.5×10^{-6} M) as the only hormone, was renewed daily. In each experiment, cells were incubated for the first 3 days with medium only and were then treated with medium containing drug at the doses and durations indicated in the individual figure legends. Drugs were added to the cultures as concentrated stock solutions in water (PB) or dimethyl sulfoxide (steroids, 0.1% of total volume).

Northern blot analysis. Total RNA was isolated from the pooled cells of three culture dishes as previously described [2]. RNA samples (20 μg) were resolved on denaturing 1% agarose gels and capillary transferred onto reinforced nitrocellulose filters (Nitro plus, MSI, Westboro, MA). cDNA inserts were radiolabeled to greater than 10^8 cpm/μg using a nick translation kit (BRL, Gaithersburg, MD) and [α-^{32}P]dCTP (3000 Ci/mmol; ICN Biomedicals, Inc., Costa Mesa, CA). Prehybridization and hybridization conditions have been described previously [16]. Final washing conditions for the cDNAs were 0.1 × standard saline citrate (20 × standard saline citrate = 3 M sodium chloride, 0.3 M sodium citrate, pH 7.5) and 0.1% sodium dodecyl sulfate at 50° (for 2B2, 2C6, TAT and β-actin) or 65° (for 3A1) for 30 min. RNA bands were visualized by autoradiography and quantified by scanning densitometry. Radioactive probes were removed from the blots by washing the filters for 15 min in water preheated to boiling before filters were rehybridized. All filters were hybridized last with the β-actin cDNA, and band intensities for 2B1/2 or 2C6 were normalized to those obtained for β-actin.

RESULTS

Preliminary experiments confirmed that in untreated, control cultures the amounts of mRNA for two constitutive P450s, 2B1/2* and 2C6, fell to undetectable levels during the first 2 days and then increased markedly between days 3 and 5 (Fig. 1). Additions of DEX to the culture medium during days 3–5 at doses of 10^{-9} and 10^{-8} M suppressed the spontaneous rise of 2B1/2 mRNA (Fig. 1). This effect was selective for 2B1/2 in that the amount of 2C6 mRNA was not suppressed by these low doses of DEX (Fig. 1). In addition, the amount of β-actin mRNA was reduced only slightly by these doses of DEX (Fig. 1). Examining the time courses of these effects (Fig. 2A), we found that, when 2B1/2 mRNA levels were not normalized to those for β-actin, treatment of hepatocyte cultures for 1 day with 10^{-8} M DEX suppressed the small increase in 2B1/2 mRNA occurring between days 3 and 4 in untreated cultures, while 2 days of 10^{-8} M DEX exposure reduced the amount of 2B1/2 mRNA to only 11.6% of that found in day 5 control cultures. When data were normalized for β-actin mRNA levels, complete suppression of the spontaneous rise of 2B1/2 mRNA was apparent after a 2-day exposure of the cultures to DEX (Fig. 2B).

The finding that DEX treatments suppressed spontaneous 2B1/2 mRNA increases at low doses suggested that this effect might be mediated through a classical GCR-mediated mechanism. In support of this idea, induction by DEX of the mRNA for TAT, a liver-specific gene product whose regulation by glucocorticoids is known to be mediated through the classical GCR [15], was maximal at 10^{-8} M DEX, in agreement with the suppressive effect of DEX on 2B1/2 mRNA (Fig. 3). In contrast, the dose–response relationship for DEX induction of P450 3A1 mRNA

* Although P450 2B2 is reported to be the major form that is expressed constitutively in rat liver, and also appears to be the form that is reexpressed spontaneously in the hepatocyte cultures, because the R17 cDNA hybridizes to both the 2B1 and 2B2 mRNAs under the conditions used for our northern blot analyses, we use 2B1/2 to describe our results. Also, the 2C6 cDNA likely cross-hybridizes to 2C7 mRNA under the northern blot conditions employed.

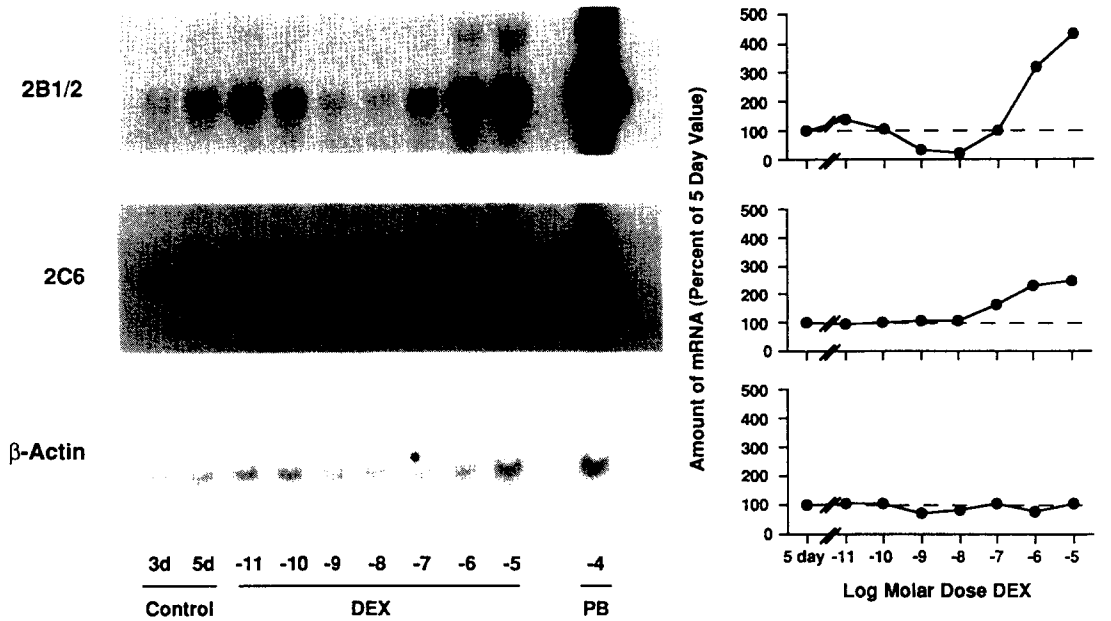

Fig. 1. Effects of DEX treatment on the amounts of P450 2B1/2, P450 2C6 and β-actin mRNAs in cultured rat hepatocytes. Freshly isolated hepatocytes prepared from the liver of a single rat were incubated for 3 days in standard, serum-free medium and then treated for 2 days with medium containing DEX at doses ranging from 10^{-11} to 10^{-5} M. Total cellular RNA was extracted and analyzed by northern blot hybridization, using cDNA probes to 2B1/2, 2C6 or β-actin, as described in Materials and Methods. For comparison, the responses produced by treatment with 10^{-4} M PB are also shown. A graph of the densitometrically quantified data, expressed as percentages of the amount of mRNA detected in 5-day-old untreated control cultures, is shown beside each autoradiograph.

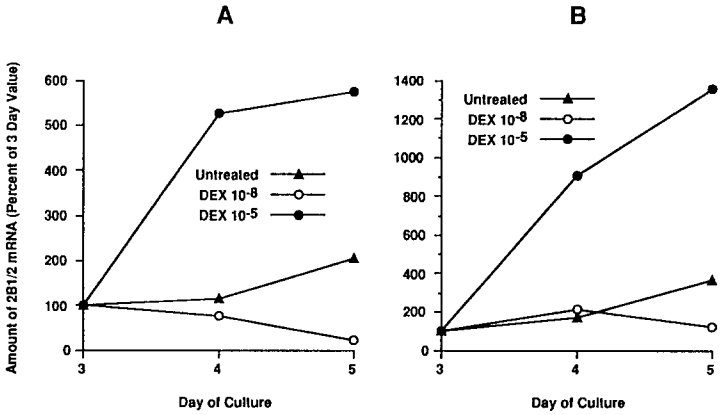

Fig. 2. Time course effects of low and high dose DEX treatments on the amount of P450 2B1/2 mRNA in cultured rat hepatocytes. Three-day-old hepatocyte cultures, prepared as described in the legend of Fig. 1, were treated with medium containing no DEX, 10^{-8} M DEX or 10^{-5} M DEX for 1 or 2 days. Total cellular RNA was extracted and analyzed by northern blot hybridization, using a cDNA to P450 2B1/2 or β-actin, and autoradiographic data were densitometrically quantified, as described in Materials and Methods. The densitometric data for 2B1/2 mRNA are shown plotted as percentages of the amount of 2B1/2 mRNA detected in untreated 3-day control cultures either without (A) or following (B) normalization to the amount of β-actin mRNA detected in the respective samples.

required higher doses of DEX (10^{-7} to 10^{-5} M) than did TAT induction (Fig. 3). We have reported previously that induction of 3A1 does not appear to be mediated through the classical GCR pathway, based on the requirement for unusually high doses of glucocorticoid and on divergent agonist–antagonist relationships [17]. Furthermore, treatment of hepatocyte cultures with low doses (10^{-9} to 10^{-8} M)

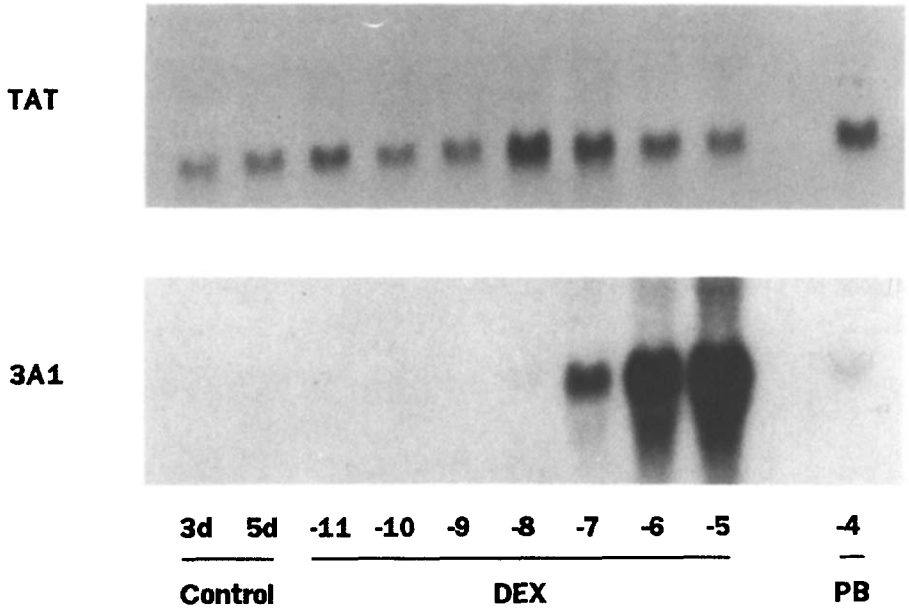

Fig. 3. Effects of DEX treatment on the amounts of P450 3A1 and TAT mRNAs in cultured rat hepatocytes. Three-day-old hepatocyte cultures, prepared as described in the legend of Fig. 1, were treated for 2 days with medium containing DEX at doses ranging from 10^{-11} to 10^{-5} M. Total cellular RNA was extracted and analyzed by northern blot hybridization, as described in Materials and Methods. The filters used to obtain the blots shown in Fig. 1 were boiled in water, to remove bound cDNA probes, and then rehybridized with cDNAs to P450 3A1 or TAT.

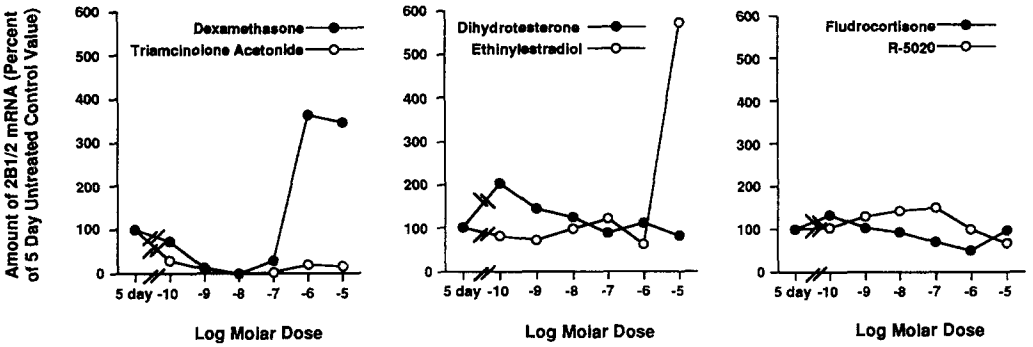

Fig. 4. Effects of treatments with various steroid classes on the amount of P450 2B1/2 mRNA in cultured rat hepatocytes. Three-day-old hepatocyte cultures, prepared as described in the legend of Fig. 1, were treated for 2 days with medium containing DEX, triamcinolone acetonide, dihydrotestosterone, 17α-ethinylestradiol, fludrocortisone or R-5020, all at doses ranging from 10^{-10} to 10^{-5} M. Total cellular RNA was extracted and analyzed by northern blot hybridization, using a cDNA to P450 2B1/2 or β-actin, as described in Materials and Methods. The autoradiographic data were densitometrically quantified, and amounts of 2B1/2 mRNA were normalized to the amounts of β-actin mRNA in the respective samples. Data are shown plotted as percentages of the amount of 2B1/2 mRNA detected in untreated 5-day control cultures.

of triamcinolone acetonide, a potent synthetic glucocorticoid that, like DEX, binds to the GCR, resulted in complete suppression of 2B1/2 mRNA (Fig. 4). Treatments with dihydrotestosterone or EE, a potent androgen and estrogen, respectively, that do not bind to the GCR, produced no definite suppression of 2B1/2 mRNA at any dose examined, although EE treatment did induce 2B1/2 mRNA at 10^{-5} M (Fig. 4). Fludrocortisone and R-5020 are a potent mineralocorticoid and progestin, respectively, although each agent also has some glucocorticoid activity [18–20]. Treatment of cultured hepatocytes with either of these steroids suppressed 2B1/2 mRNA only at high doses (10^{-6} to 10^{-5} M) (Fig. 4).

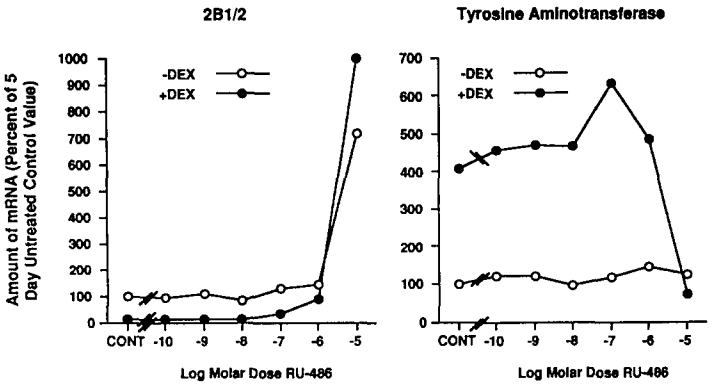

Fig. 5. Northern blot analysis of P450 2B1/2 and TAT mRNAs in cultured rat hepatocytes cotreated with DEX and RU-486. Three-day-old hepatocyte cultures, prepared as described in the legend of Fig. 1, were treated for 2 days with medium containing RU-486 at doses ranging from 10^{-10} to 10^{-5} M in the presence or absence of 10^{-8} M DEX. Total cellular RNA was extracted and analyzed by northern blot hybridization, using a cDNA to P450 2B1/2, TAT or β-actin, as described in Materials and Methods. The autoradiographic data were densitometrically quantified, and amounts of 2B1/2 or TAT mRNA were normalized to the amounts of β-actin mRNA in the respective samples. Data are shown plotted as percentages of the amount of 2B1/2 (left) or TAT (right) mRNA detected in untreated 5-day control cultures.

We attempted to reverse suppression of 2B1/2 mRNA by cotreating the cultured hepatocytes for 2 days with 10^{-8} M DEX plus various doses of RU-486, a potent antiglucocorticoid [21] (Fig. 5). Treatment of the cultures with RU-486 alone had essentially no effect on the amount of 2B1/2 mRNA at doses from 10^{-10} to 10^{-6} M, but induced 2B1/2 mRNA at 10^{-5} M. RU-486 was unable to reverse the DEX-mediated suppression of 2B1/2 mRNA at doses that did not themselves induce 2B1/2 mRNA, while higher doses of RU-486 in combination with DEX resulted in the same induction seen with RU-486 alone. Rehybridization of the northern filter with the TAT cDNA revealed that effective inhibition of TAT mRNA induction occurred only at the same dose of RU-486 (10^{-5} M) that by itself induced 2B1/2. The inductive effect of RU-486 alone, therefore, precluded us from obtaining additional information about the involvement of the classical GCR in 2B1/2 mRNA suppression by DEX.

In view of the finding that DEX suppressed the spontaneous increase of 2B1/2 mRNA, we examined the effect of this steroid on the drug-induced increase in 2B1/2 mRNA produced by PB. Cotreating the cultures for 2 days with 10^{-4} M PB, a dose we have found previously to be optimum for 2B1/2 induction [3], and a range of DEX doses (10^{-8} to 10^{-5} M) (Fig. 6), we found that 2B1/2 mRNA induction was not suppressed, but rather was potentiated by low doses of DEX (2.5-fold by 10^{-7} M DEX). This effect was reversed upon incubations with higher doses of DEX.

Treatment of the cultures with higher doses of DEX produced opposite, nonsuppressive effects on the spontaneous rise in 2B1/2 mRNA. Treatment of the cultures with DEX at 10^{-6} and 10^{-5} M resulted in greater amounts of 2B1/2 mRNA (4.1-fold at 10^{-5} M) when compared with the 2B1/2 mRNA levels in 5-day incubated control cultures (Fig. 1). The level of 2B1/2 mRNA induction by 10^{-5} M DEX showed a time-dependent increase throughout 2 days of treatment (Fig. 2). DEX at high doses (10^{-6} and 10^{-5} M) also increased the amount of the 2C6 mRNA (up to 2.4-fold relative to the 5-day control value), but had no effect on the amount of β-actin mRNA (Fig. 1). The doses of DEX that induced the 2B1/2 and 2C6 mRNAs were identical to those that effectively induced 3A1 mRNA (Fig. 3). These findings suggested that the induction of 2B1/2 mRNA by high doses of DEX was not regulated through a classical glucocorticoid pathway, similar to our previous findings for 3A1 [17]. In support of this idea, treatment of hepatocyte cultures with high doses of the potent glucocorticoid triamcinolone acetonide did not induce 2B1/2 mRNA (Fig. 4), whereas treatment with the antiglucocorticoid RU-486 did induce 2B1/2 mRNA (Fig. 5).

DISCUSSION

DEX treatment affects 2B1/2 gene expression in living rats, but the patterns of regulation appear to be complex. For example, Simmons *et al.* [9] reported that DEX treatment (80 mg/kg) of Sprague–Dawley rats elevates the level of hepatic 2B1/2 mRNA but decreases the amount of 2B1 protein and produces no change in gene transcription. Rao *et al.* [10] found that DEX treatment (50 mg/kg) potentiates induction of 2B1/2 mRNA by PB in rats, but inhibits transcription, and suggested that DEX stabilizes the primary nuclear transcripts. In this study, we have taken advantage of our finding that primary cultures of rat hepatocytes maintained on dishes coated with Matrigel spontaneously reexpress the mRNAs for several constitutive P450s, including those for 2B1/2 and 2C6 [8]. We critically examined the effects of

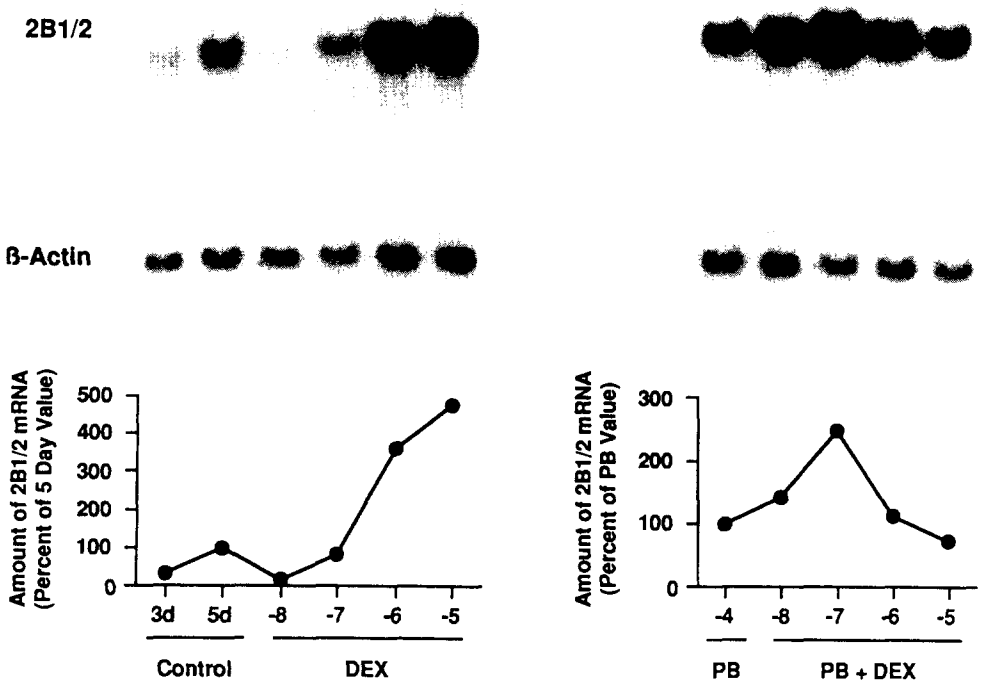

Fig. 6. Effects of DEX treatment on spontaneous and PB inducible expression of P450 2B1/2 mRNA in cultured rat hepatocytes. Three-day-old hepatocyte cultures, prepared as described in the legend of Fig. 1, were treated with medium containing doses of DEX ranging from 10^{-8} to 10^{-5} M for 2 days in the absence (left) or presence (right) of 10^{-4} M PB. Total cellular RNA was extracted and analyzed by northern blot hybridization, using a cDNA probe to P450 2B1/2 or β-actin, as described in Materials and Methods. A graph of the densitometrically quantified 2B1/2 mRNA data, normalized to the amounts of β-actin mRNA and expressed as percentages of either the amount of 2B1/2 mRNA detected in 5-day-old untreated control cultures (left) or in cultures treated with 10^{-4} M PB alone (right), is shown beneath each respective autoradiograph. The blots for 2B1/2 mRNA in the absence (left) or presence (right) of PB were exposed to X-ray film for 1 day or 3 hr, respectively.

DEX treatment on the mRNA levels of these forms, and found that treatment of the cultured hepatocytes with low doses of DEX (10^{-9} to 10^{-8} M) selectively suppressed 2B1/2 mRNA levels in a manner generally consistent with a classical GCR-mediated mechanism, whereas high doses (10^{-6} to 10^{-5} M) induced the 2B1/2 and 2C6 mRNAs. Although decreased levels of other mRNAs, for example procollagen mRNA [22], have been reported previously in primary rat hepatocytes cultured in the presence of low doses of DEX, to our knowledge this is the first example of a dose-dependent biphasic effect by DEX on gene expression.

Hepatocytes rapidly lose their P450 mRNA content following placement of the cells into primary culture. Explanations for this loss are not established, but likely include an inability of the cells to transcriptionally activate the P450 genes early in culture [7, 23], which could result from damage incurred by the hepatocytes during the isolation and culture procedures. The increases in the levels of certain P450 mRNAs that spontaneously occur later in culture may then simply be attributable to repair of this damage and restoration of normal intrahepatocellular conditions. If damage and repair account for the loss and subsequent restoration of constitutive P450 levels, it may follow that suppression of 2B1/2 mRNA expression upon treatment with low doses of DEX results from interference with cellular repair processes. However, in these experiments treatment with DEX was initiated on day 3 of culture, and our previous results show that the 2B1/2 and 2C6 mRNAs have begun increasing after day 2 [8], suggesting that "repair" has become advanced by this time. Also, a general mechanism, such as prevention of repair, does not readily explain the specificity for suppression of 2B1/2 mRNA, as demonstrated by the failure of low dose DEX treatment to affect 2C6 mRNA levels.

Our results therefore appear to be more consistent with a specific effect of DEX on expression of the 2B1/2 genes. In this regard, Jaiswal *et al.* [24] recently described a glucocorticoid responsive region in the 5′ flanking region of the 2B2 gene. However, this element was found to confer DEX inducibility (at 10^{-7} M DEX), rather than suppression, upon a reporter gene in stably transfected hepatoma cells. Although many of the steps in the glucocorticoid

gene activation pathway have been elucidated [25–27], suppression by glucocorticoids is poorly understood. Current evidence suggests that suppression may be mediated by mechanisms whereby the GCR binds to (1) a negative GRE, (2) a GRE thereby preventing binding to the gene of a second positive transcriptional factor, or (3) a positive transcriptional factor itself, thereby preventing this factor from stimulating gene transcription [25–27]. As an example of the latter mechanism, glucocorticoid suppression of several genes that are transcriptionally activated by the Fos/Jun complex has been attributed to direct binding of the glucocorticoid receptor to Fos, thereby preventing binding of Fos/Jun to AP1 sites on the target genes [28].

Our results also demonstrated that low doses of DEX increased, rather than suppressed, PB-inducible expression of 2B1/2, consistent with some recent reports [23, 29, 30]. Reversal of potentiated PB induction at higher doses of DEX has also been reported recently [31]. Low doses of DEX, therefore, appear to exert fundamentally different effects on spontaneous versus inducible expression of 2B1/2 mRNA, possibly reflecting the presence in the gene of multiple DEX responsive regions or differences in the function of a single DEX responsive region depending on whether or not inducer is present.

An additional complexity in the regulation of 2B1/2 expression by DEX was revealed by the finding that high doses of DEX (10^{-6} to 10^{-5} M) induced, rather than suppressed, the mRNAs for 2B1/2 and 2C6 in the cultured hepatocytes. These inductive effects were not consistent with a classical glucocorticoid receptor-mediated effect in that triamcinolone acetonide, a potent glucocorticoid, failed to induce 2B1/2 mRNA at high doses, whereas RU-486, a potent antiglucocorticoid, did induce 2B1/2 mRNA at these doses. Consistent with findings in treated rats [9], the inductive effect on 2B1/2 mRNA by high dose DEX treatment was relatively modest compared to the induction of 2B1/2 by PB or of 3A1 by DEX. In contrast, DEX has been shown to be an efficacious inducer of the mouse 2B orthologue [32].

Our previous findings in rat hepatocytes cultured on Matrigel have demonstrated that this culture system accurately reproduces many of the patterns of inducible P450 expression seen in the livers of rats [2, 5]. If this fidelity extends to include similarities between the mechanisms allowing the spontaneous increase of 2B1/2 mRNA in primary cultured rat hepatocytes and those regulating basal expression of 2B2 *in vivo*, then low doses of glucocorticoids would be expected to suppress basal 2B2 expression in rat liver. Normal plasma concentrations of corticosterone, the major glucocorticoid in rats, are reported to range between approximately 10^{-8} and 7×10^{-7} M [33, 34], which, considering the greater potency of DEX, should be near the range expected to suppress basal 2B1/2 expression. Since 2B2 is present in untreated rat liver, there must be countervailing factors active in the living animal that allow constitutive expression of the 2B2 gene to occur in the presence of a suppressive influence. Precedent for this is provided by our previous observations that continuous incubation of hepatocyte cultures with physiological doses of growth hormone, mimicking the pattern of growth hormone secretion occurring in the female rat, completely abolished the ability of PB to induce 2B1/2 mRNA [35], despite the fact that PB treatment induces 2B1/2 in female rats.

We undertook this series of studies with the expectation that the levels of expression of constitutive P450s would be maintained by multifactorial regulatory controls. Our findings that low doses of glucocorticoids suppressed the spontaneous increase of 2B1/2 mRNA in cultured rat hepatocytes whereas high doses of DEX induced 2B1/2 mRNA provide further evidence that DEX affects 2B1/2 expression through multiple regulatory mechanisms. It is known that other hormones, such as growth hormone [36, 37], thyroid hormone [38] and insulin [37], can exert effects on the levels of these P450 forms in the basal steady state. Moreover, even for a single factor like DEX, studied in isolation, dose proves to be an important variable. While more work lies ahead to identify the native factors controlling P450 expression, the availability of a hepatocyte culture system that vigorously expresses the constitutive P450s provides a crucial tool to allow these issues to be addressed.

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