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EFFECT OF DEXAMETHASONE ON CYTOCHROME P-450 MEDIATED METABOLISM OF 2-ACETYLAMINOFLUORENE IN CULTURED RAT HEPATOCYTES

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Abstract—The metabolism of 2-acetylaminofluorene (AAF) to its six oxidative metabolites has been used to investigate the effect of dexamethasone on cytochrome P-450 activity in cultured rat hepatocytes. In control hepatocytes the metabolism of AAF to its 1-, 5-, 7-, 9- and *N*-hydroxylated metabolites rapidly declined in culture over the first 24 hr while 3-hydroxylation remained relatively constant. These activities either remained unchanged or increased slightly during the next 48 hr in culture. The addition of dexamethasone (100 nM) to the culture medium had little effect in arresting the initial decline but by 72 hr the 7-, 5- and 3-hydroxylations increased to values 2.5, 16 and 21 times the respective 24-hr values. The inductive effect of dexamethasone on the 3- and 5-hydroxylations of AAF was maximal at 100 nM whereas the 7-hydroxylation increased linearly as a function of the dexamethasone concentration up to 1 μM. Cortisol and corticosterone and the non-glucocorticoids fluoxymesterone and methyltestosterone induced a pattern of AAF metabolism resembling that in dexamethasone-treated cultures, suggesting that a range of steroids not restricted to glucocorticoids may induce multiple cytochrome P-450 isozymes via related mechanisms. Pregnenolone 16α-carbonitrile induced only the 7-hydroxylation of AAF probably reflecting induction of cytochrome P-450p. While dexamethasone was a strong inducer of the 3- and 5-hydroxylations of AAF in hepatocyte culture, assay of these activities in freshly isolated cells after *in vivo* treatment with dexamethasone showed a strong induction of 7-hydroxylation but only small effects on 3- and 5-hydroxylations. Indeed the profile of AAF metabolism induced in culture by dexamethasone resembles more closely the profile induced by 3-methylcholanthrene *in vivo*. These data suggest that factors yet to be identified strongly influence the steroid-induced pattern of cytochrome P-450 gene expression.

The hepatic microsomal cytochrome P-450 mono-oxygenases are the major enzymes involved in the metabolism of most lipophilic drugs and chemical carcinogens. These enzymes also metabolise a variety of endogenous substrates such as steroids, fatty acids, biogenic amines and prostaglandins [1, 2]. An important property of this mono-oxygenase system is the differential inducibility of different isoenzymes of cytochrome P-450. Traditionally, inducers of cytochromes P-450 have been classified as either phenobarbital-like or 3-methylcholanthrene-like. However, recent observations have suggested that glucocorticoids such as dexamethasone and some further steroids such as pregnenolone 16α-carbonitrile (PCN*), constitute a 'third' class of inducers [3–5]. Both dexamethasone and PCN have been shown to induce the same form of cytochrome P-450 (P-450p) and this hemoprotein is biochemically and immunochemically distinct from members of the phenobarbital or 3-methylcholanthrene responsive families [6, 7]. While only a single glucocorticoid-PCN inducible cytochrome P-450 and cDNA have been isolated and characterised, indications are that a family of P-450p proteins may exist [3, 4]. There is so far only limited information [3, 6, 8–10] on how P-450p and any related steroid-inducible isozymes are controlled.

Various metabolic activities such as ethylmorphine [7] and erythromycin [3] *N*-demethylases, mephenytoin 4-hydroxylase [11] and triacetyloleandomycin complex formation with P-450p [3, 12] have been used to estimate the amount of P-450p in microsomes. However, good correlations between these activities and immunoreactive P-450p have not always been obtained, possibly indicating that P-450 isoenzymes other than P-450p contribute to these reactions [3, 8, 11]. Recently we have shown that the proposed equivalent of P-450p in rabbit liver microsomes, Form 3c [3], selectively hydroxylates 2-acetylaminofluorene (AAF) in the 7 position [13], while Astrom and De Pierre [14] found that the purified rat P-450p hydroxylated AAF in the 7 and N positions. These data clearly indicate that AAF is a substrate of this glucocorticoid-inducible cytochrome P-450. Further, AAF is metabolised to six different oxidative metabolites and these appear to be catalysed by several distinct forms of cytochrome P-450 in rat [15], rabbit [13] and human [16] liver

* Abbreviations: AAF, 2-acetylaminofluorene; PCN, pregnenolone 16α-carbonitrile; TAT, tyrosine amino transferase.

microsomes. Thus the metabolism of this substrate, besides providing a means of monitoring P-450p activity, should also enable the detection of the effect of glucocorticoids on pathways mediated by other forms of cytochrome P-450. To gain further insight into the role of glucocorticoids and related steroids in mono-oxygenase regulation we have used AAF as a probe to study cytochromes P-450 induction in cultured rat hepatocytes. Primary cultures of hepatocytes were chosen as a model since these provide cells under fully defined, readily manipulated conditions which, with appropriate choice of media, may exhibit a range of responses resembling those of cells *in vivo* [9, 17, 18]. Since it is apparent that expression of cytochrome P-450 isozymes in culture is dependent on culture conditions [9, 10, 17, 19] and may differ from expression *in vivo* [17, 19–21], we have initially compared findings on control of AAF metabolism in cultured cells with the effects of steroid and other inducers *in vivo*.

METHODS

Chemicals. Randomly labelled [^{3}H]AAF (18 Ci/mmol) was purchased from Moravek Biochemicals (Brea, CA) and was purified to >98% purity by HPLC [22]. Unlabelled AAF and 2-aminofluorene were obtained from Eastman Organic Chemicals (Rochester, NY). Authentic N-OH-AAF, 1-OH-AAF, 3-OH-AAF, 5-OH-AAF, 7-OH-AAF, 9-OH-AAF, 2-acetylaminofluorene-9-one and AAF were generously donated by Dr Snorri S. Thorgeirsson, National Cancer Institute, U.S.A. Desferyloxamine mesylate was purchased from Ciba Pharmaceuticals (Summit, NJ) and all other biochemicals were purchased from Sigma Chemical Co. (St. Louis, MO) except collagenase which was from Boehringer Mannheim (Australia). Sources and formulations of media were as previously described [23].

Animals. Male Porton strain Wistar rats weighing 200–300 g were obtained from the Institute of Medical and Veterinary Science (Adelaide, Australia) and were housed in plastic cages and allowed free access to food and water. For some studies, rats were treated (i.p.) with sodium phenobarbital (in normal saline: 75 mg/kg) once daily for 4 days, dexamethasone sodium phosphate (in normal saline; 100 mg/kg) once daily for 4 days, or 3-methylcholanthrene (in corn oil; 30 mg/kg) once daily for 3 days prior to sacrifice.

Hepatocyte isolation and culture. Hepatocytes were isolated by a two-step collagenase perfusion procedure modified to yield sterile preparations [23]. The procedures for cell culture were essentially as in ref. [19]. Briefly, confluent cultures were established by allowing 2.8×10^6 freshly isolated hepatocytes to attach to collagen-coated, 60-mm-diameter culture dishes in modified Waymouth medium [23] with 10 mM nicotinamide and 3% foetal calf serum (Commonwealth Serum Laboratories, Melbourne, Australia). After allowing 3 hr for cell attachment, the medium was changed to a standard defined 'culture medium' which, unless otherwise specified, was Williams medium E supplemented with 10 mM nicotinamide and the antibiotics penicillin G (60 μg/ml) and streptomycin sulphate (100 μg/ml). Test compounds dissolved in 2.5 μl dimethyl sulphoxide were added to cultures at this time, and thereafter with daily medium changes. Control cultures received the vehicle only.

2-Acetylaminofluorene metabolism in intact hepatocytes. After experimental pre-treatments, hepatocyte monolayers were washed twice with warm 0.15 M NaCl. [^{3}H]AAF (50 μM) was then added to the monolayer cultures in 1.5 ml of Krebs–Henseleit buffer and incubated for 20 min. The reaction was stopped by removing the media to a test tube and adding 1 ml of ice-cold sodium acetate (1 M, pH 6.0) to the cells prior to placing them on ice. The cells were then scraped free and the cell suspension combined with the media fraction above. Each dish was washed a further time with 1 ml of sodium acetate and then the combined aqueous layers were extracted with 10 ml of ice-cold ether. Authentic standards of AAF and its metabolites were added to the sodium acetate solution as carriers and to visualise absorbance peaks during chromatography. After one ether extraction, the remaining aqueous phase was incubated overnight with β-glucuronidase (5000 units) and sulphatase (12 units). Additional standards of AAF and metabolites were then added to the incubation mixture prior to extracting twice more with 10 ml of ether. The three ether extracts were pooled, filtered and evaporated to dryness under nitrogen, and residues were dissolved in 0.1 ml of methanol for chromatographic analysis. When AAF metabolism was determined in freshly isolated cells $0.1–0.5 \times 10^6$ hepatocytes were incubated with 50 M AAF for 5 min and the reaction was stopped with 1 ml of the above ice-cold sodium acetate solution. AAF and its metabolites were then extracted as outlined above. AAF metabolised in either cultured or freshly isolated cells did not exceed 10% of the substrate added. The DNA content of the aqueous layer was determined by the method of Erickson *et al.* [24].

Chromatography. AAF and its metabolites were determined according to the method of Smith and Thorgeirsson [22]. Briefly, 10 μl of the reconstituted sample were injected onto a DuPont Zorbax C_8 (4.6 mm × 15 cm) column. A flow rate of 1.5 ml/min was used, and absorbance was monitored at 280 nm. The initial solvent was 23% isopropyl alcohol: 77% 0.01 M acetic acid; both solutions contained 0.01% desferrioxamine mesylate to prevent chemisorption of *N*-hydroxy-AAF and to provide the best resolution of AAF and 8 of its metabolites [22]. Instrumentation consisted of a Beckman Model 342 high-pressure liquid chromatograph, two Beckman Model 114M pumps, a Beckman Model 420 microprocessor, a Beckman model 160 detector, and Kipp and Zonen BD40 recorder.

Assays of tyrosine amino transferase (TAT) and protein. For TAT assays hepatocyte monolayers were washed twice with 0.15 M NaCl and scraped from dishes into 2-ml 0.1M potassium phosphate pH 7.5, containing 1 mM EDTA and 0.1% Triton X-100. Samples were homogenised for 10 sec with an Ultraturrax homogeniser and TAT activity determined by the method of Diamondstone [25]. Protein was determined by the Lowry procedure [26].

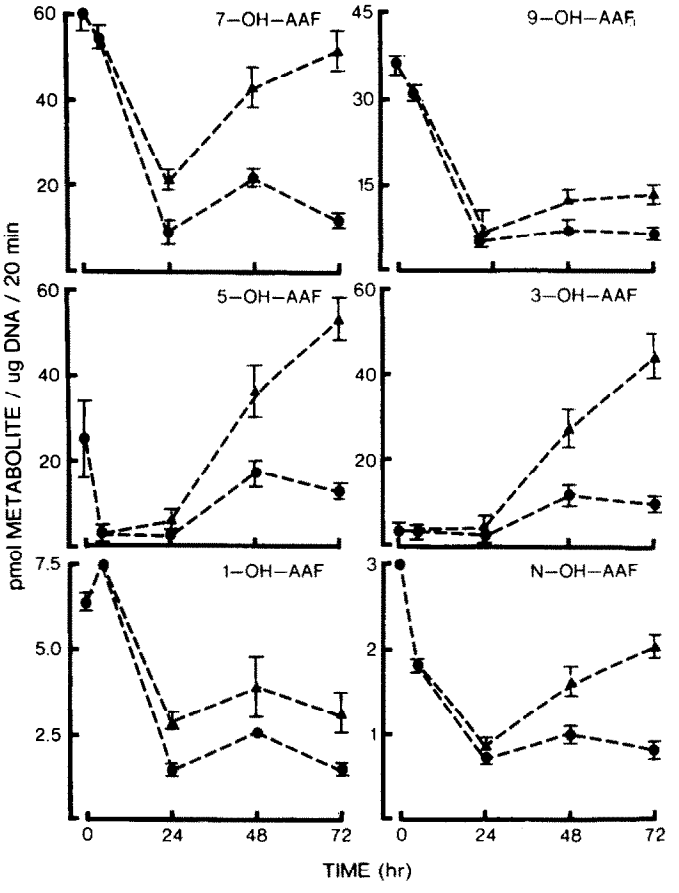

Fig. 1. Effect of time in culture on AAF to its six oxidative metabolites in control (●) and 100 nM dexamethasone (▲) treated hepatocytes. Each point represents mean ± SD of at least three dishes.

RESULTS

Figure 1 shows changes with time in culture, in rates of site-specific AAF hydroxylations when hepatocytes were maintained for up to 72 hr in defined culture medium with or without dexamethasone. During the first 24 hr in culture all metabolic pathways except for 3-hydroxylation of AAF declined rapidly to values 40% or less of the activities in freshly isolated cells. This initial decline was only slightly affected by addition of dexamethasone. Compared with 24-hr values, all metabolic activities either remained unchanged or increased slightly in the succeeding 48 hr in control cultures. In the presence of dexamethasone (100 nM) the 7-, 5- and 3-hydroxylations of AAF had increased by 72 hr to values 2.5, 16 and 21 times the respective 24-hr activities, and 5, 4.5 and 4 times the corresponding activities in 72-hr control cultures. There was also a slight but significant increase in the rates of 1-, 9- and *N*-hydroxylations of AAF by 72 hr. Only in the case of the 3- and 5-hydroxylations of AAF were the 72-hr activities significantly greater than the same activities determined in freshly isolated cells. The rate of 3-hydroxylation of AAF in cultured hepatocytes at 72 hr was 16-fold higher than freshly isolated cells whereas the 5-hydroxylation was 2-fold higher.

Figure 2 shows dose–response curves for the induction of AAF metabolism by dexamethasone in hepatocytes maintained in culture for 72 hr. Both the 7- and 9-hydroxylations of AAF increased linearly as a function of the dexamethasone concentration up to 1 μM. In the case of the 3- and 5-hydroxylations of AAF the induction was maximal at lower dexamethasone concentrations (30 nM). Induction of AAF *N*-hydroxylation also appeared to be maximal at 30–100 nM dexamethasone, whereas the 1-hydroxylation increased only marginally as a function of the steroid concentration. Under the same conditions TAT, a glucocorticoid responsive enzyme [27, 28] was induced 6-fold and the inductive effect of dexamethasone on this activity was maximal at 10 nM (Table 1).

The effect on AAF metabolism of treating hepatocytes in culture for 72 hr with other glucocorticoids, non-glucocorticoids and antiglucocorticoids was also investigated (Tables 2 and 3). Cortisol and corticosterone both induced AAF metabolism in a manner similar to the dexamethasone-induced pattern but substantially higher concentrations (50 μM) of these endogenous glucocorticoids were required. Even at 50 μM, the induction of TAT by these compounds was suboptimal (Table 1), as indicated by comparison with the TAT-inducing action of 30–100 nM

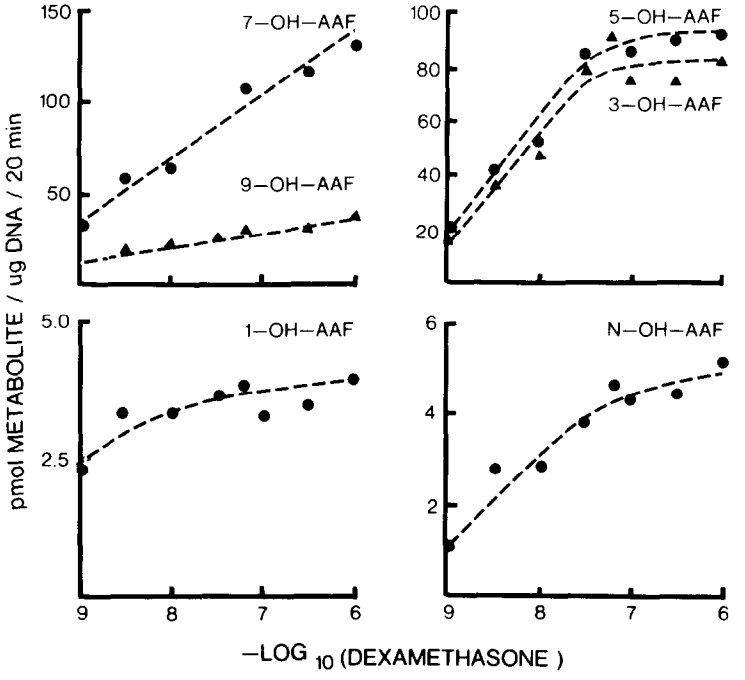

Fig. 2. Concentration dependence of induction of AAF metabolism by dexamethasone in rat hepatocytes maintained in culture for 72 hr. Values represent the mean of duplicate determinations.

dexamethasone, possibly reflecting rapid metabolism of natural glucocorticoids in hepatocytes. PCN, a catatoxic steroid which has been shown to induce a specific form of cytochrome P-450 *in vivo* and in cultured rat hepatocytes [7, 8], induced only the 7-hydroxylation of AAF. Treatment of hepatocytes with progesterone, methyltestosterone or fluoxymesterone resulted in a significant increase in the rates of 7-, 9-, 5- and 3-hydroxylations of AAF compared to corresponding control values (Table 3). Indeed, fluoxymesterone at a concentration of 50 μM was as effective as dexamethasone (100 nM) in inducing AAF metabolism. In combination with dexamethasone, fluoxymesterone produced an additive inductive effect on the rates of 7- and 3-hydroxylations of AAF. The combination of progesterone and dexamethasone resulted in activities similar to those seen in the absence of dexamethasone whereas methyltestosterone had no effect on the capacity of this glucocorticoid to induce AAF metabolism in cultured rat hepatocytes, except for a minor effect on the 1-hydroxylation pathway. When the effect of fluoxymesterone, methyltestosterone and progesterone on TAT activity in hepatocytes was investigated none of the compounds alone induced the enzyme (Table 1). All three compounds have been reported

Table 1. Effects of steroids on tyrosine aminotransferase activity in cultured rat hepatocytes

Treatment	Concentration	Tyrosine aminotransferase activity (pmol/mg protein/mg)
None	—	13.0 ± 1.6
Dexamethasone	10 nM	78.9 ± 4.7
	100 nM	84.3 ± 4.9
PCN	10 μM	15.4 ± 4.7
Cortisol	50 μM	35.2 ± 5.0
Corticosterone	50 μM	34.1 ± 2.1
Fluoxymesterone	50 μM	12.6 ± 1.3
Methyltestosterone	50 μM	12.6 ± 1.3
Progesterone	50 μM	10.8 ± 1.4

Hepatocytes were incubated in monolayer cultures for 3 days in medium containing the indicated steroids and tyrosine aminotransferase activities were determined as described in Materials and Methods. Values represent mean ± SD from four dishes.

Table 2. Effects of steroids on 2-acetylaminofluorene metabolism in cultured rat hepatocytes

Treatment	Formation of AAF metabolites (pmol/μg DNA/20 min)					
	7-OH-AAF	9-OH-AAF	5-OH-AAF	3-OH-AAF	1-OH-AAF	N-OH-AAF
None	23 ± 2	10 ± 1	24 ± 3	19 ± 2	1.7 ± 0.4	1.0 ± 0.6
Dexamethasone (100 nM)	44 ± 6	12 ± 2	47 ± 9	38 ± 7	2.4 ± 0.4	1.7 ± 0.4
Cortisol (50 μM)	48 ± 8	14 ± 2	49 ± 8	38 ± 6	2.7 ± 0.3	2.0 ± 0.3
Corticosterone (50 μM)	49 ± 5	16 ± 2	41 ± 4	33 ± 4	2.5 ± 0.6	1.8 ± 0.3
PCN (10 μM)	37 ± 6	13 ± 2	29 ± 4	22 ± 3	2.1 ± 0.3	1.3 ± 0.2

AAF metabolism was measured in hepatocyte monolayers after maintenance for 72 hr in culture with the added steroids shown. Values represent mean ± SD from three dishes.

to have antiglucocorticoid activity in hepatoma cells [10, 27, 28] but at 50 μM only progesterone could be shown to cause partial antagonism of TAT (data not shown) and AAF induction by dexamethasone. Relative insensitivity of primary hepatocytes (compared with hepatoma cells) to antiglucocorticoids has been reported previously [29].

To allow direct comparison of patterns of AAF metabolism (with or without exposure to glucocorticoid) in culture with patterns after treatment with P-450 inducers *in vivo*, the profile of AAF metabolism was determined in freshly isolated hepatocytes from animals pre-treated with 3-methylcholanthrene, phenobarbital or dexamethasone. All three *in vivo* treatments dramatically changed the metabolic profile of AAF compared to controls (Table 4). 3-Methylcholanthrene caused marked increases in hydroxylation at all positions with the largest relative effects on 3-, 5- and *N*-hydroxylations. Phenobarbital markedly increased the 7- and 9-hydroxylations with smaller relative effects on the other positions. The effect of dexamethasone *in vivo* differed from either 3-methylcholanthrene or phenobarbital, with the largest effect on the 7-hydroxylation pathway. Dexamethasone, like 3-methylcholanthrene, also caused an increase in the *N*-hydroxylation of AAF, the metabolic activation pathway. In cultured cells (Tables 2, 3) dexamethasone induced 7-, 9- and *N*-hydroxylations, but to a much smaller extent than *in vivo* and strongly induced the 3- and 5-hydroxylation pathways *in vitro*. The pattern of AAF metabolism induced by dexamethasone in culture was more similar (qualitatively) to the pattern of activities induced by 3-methylcholanthrene *in vivo* than to the *in vivo* dexamethasone-induced pattern.

DISCUSSION

The present investigation has used the metabolism of AAF to its six oxidative metabolites to study cytochrome P-450 activity in cultured rat hepatocytes. Except for the 3-hydroxylation, the metabolism of AAF (to its 1-, 5-, 7-, 9- and *N*-hydroxylated metabolites) rapidly declined in control hepatocytes over the first 24 hr in culture. These results confirm previous observations using other substrates that most cytochrome P-450 activities of hepatocytes undergo an initial and rapid decline in culture when maintained in unsupplemented culture media [17–19, 21]. The addition of dexamethasome to the culture medium had very little effect in arresting this decline, but by 72 hr in culture marked increases in the rates of production of individual metabolites of AAF were observed in the presence of this glucocorticoid.

Dexamethasone or PCN have been shown by Guzelian and co-workers [6, 8] to induce the same

Table 3. Effect of antiglucocorticoids on the induction of 2-acetylaminofluorene metabolism by dexamethasone in cultured rat hepatocytes

Treatment	Formation of AAF metabolites (pmol/μg DNA/20 min)					
	7-OH	9-OH	5-OH	3-OH	1-OH	N-OH
None	13 ± 7	4 ± 1	15 ± 8	7 ± 4	2 ± 1	1 ± 0.5
Dexamethasone (100 nM)	55 ± 6	14 ± 1	58 ± 8	38 ± 3	4 ± 1	2 ± 0.3
Progesterone (50 μM)	34 ± 7	8 ± 1	23 ± 5	15 ± 3	2 ± 0.7	1 ± 0.4
Dexamethasone + progesterone	39 ± 10	11 ± 3	28 ± 12	20 ± 9	1 ± 0.3	0.6 ± 0.2
Fluoxymestrone (50 μM)	51 ± 4	12 ± 1	59 ± 3	36 ± 2	4 ± 1	2 ± 1
Dexamethasone + fluoxymestrone	70 ± 6	24 ± 15	67 ± 8	50 ± 4	3 ± 0.4	2 ± 0.2
Methyltestosterone (50 μM)	30 ± 4	10 ± 2	33 ± 1	22 ± 5	2 ± 0.3	1 ± 0.3
Dexamethasone + methyltestosterone	60 ± 2	14 ± 1	57 ± 6	39 ± 4	2 ± 0.3	2 ± 0.1

Values represent mean ± SD from three dishes. AAF metabolism was measured in hepatocyte monolayers after maintenance for 72 hr in culture with the added steroids shown.

Table 4. 2-Acetylaminofluorene metabolism in freshly isolated hepatocytes from control and induced rats

Treatment	Formation of AAF metabolites (pmol/μg DNA/20 min)					
	7-OH-AAF	9-OH-AAF	5-OH-AAF	3-OH-AAF	1-OH-AAF	N-OH-AAF
Corn oil	49 ± 4	35 ± 2	12 ± 13	2.3 ± 0.4	5.4 ± 1.1	2.2 ± 1.2
Dexamethasone	618 ± 66	297 ± 34	19 ± 2	17 ± 2	12 ± 2	12 ± 2
Phenobarbital	343 ± 19	494 ± 33	48 ± 22	32 ± 6	45 ± 3	ND
3-Methylcholanthrene	665 ± 100	250 ± 65	650 ± 35	630 ± 72	29 ± 3	77 ± 14

Rats were treated with the compounds shown for 3 or 4 days as in Materials and Methods, hepatocytes isolated and AAF metabolism measured in suspensions of freshly isolated cells. Values represent mean ± SD of duplicate determinations on at least three animals.

form of cytochrome P-450 (P-450p) *in vivo* and *in vitro*. Consistent with these observations, 7-hydroxylation of AAF which is considered to be one metabolic activity of P-450p [14], was induced by dexamethasone *in vivo* (Table 4) and also in hepatocytes in primary culture (Figs 1, 2; Tables 2, 3). PCN also induced 7-hydroxylation about 2-fold in cultured cells (Table 2). P-450p is also capable of *N*-hydroxylating AAF [14] and in this study dexamethasone increased the *N*-hydroxylation 5-fold *in vivo* (Table 4), and about 2-fold in culture (Figs 1, 2; Table 3), although in culture the level of *N*-hydroxylation was close to the detection limit.

The present findings on changes in AAF 7-hydroxylation as a putative measure of P-450p resemble previous observations with cultured hepatocytes where ethylmorphine *N*-demethylase activity [8] or immunochemical techniques [8, 9] were used as measures of P-450p levels. In both studies indicators of P-450p levels declined during the first 24 hr in culture and were thereafter inducible by PCN or dexamethasone. When measured immunochemically, synthesis of P-450p increased more than 20-fold after dexamethasone addition [9]. The smaller relative changes observed in ethylmorphine demethylase [8] and in AAF 7-hydroxylation (this study) compared to immuno-reactive protein may reflect the induction of P-450 isozymes that show similar epitopes but have differing substrate specificities. Indeed, while only a single glucocorticoid-PCN-inducible cytochrome P-450 has been isolated and characterised, indications are that a family of P-450p proteins may exist [3, 4]. Alternatively this may also indicate an excess of apoprotein over the availability of heme prosthetic groups to form holocytochromes P-450 in the cultured cells.

From the comparison of dexamethasone-induced changes in hepatocyte 7-hydroxylation of AAF *in vivo* and in culture, it is apparent that under the simple culture conditions so far tested, much smaller inductions were observed *in vitro* than *in vivo*. Furthermore, measurements of AAF hydroxylation at several positions provided a measure of changes in levels of a variety of P-450 isozymes. From the comparison of *in vivo* and *in vitro* changes it appears that while dexamethasone was a weaker inducer of P-450p in culture it probably caused induction of several other isozymes. It was a strong inducer of 3- and 5-hydroxylations, activities affected only slightly by dexamethasone in the liver *in vivo*. According to Astrom *et al.* [14] the major rat P-450 isozymes contributing to 3- and 5-hydroxylating activities are P-450c and P-450d. Thus these observations on AAF metabolism are consistent with the view that under the culture conditions used, dexamethasone favoured induction of some forms of P-450 inducible by polycyclic hydrocarbons such as 3-methylcholanthrene. Previous reports have suggested that glucocorticoids have a broad effect in maintaining cytochrome(s) P-450 and associated monooxygenase activities in culture [19, 30, 31] and that in general such cultures express predominantly polycyclic hydrocarbon-responsive forms of P-450 [17–19, 30–33]. There is so far little information on why the conditions of cell culture favour an altered pattern of gene expression in response to inducers such as dexamethasone. It has been shown that media supplements may partly restore inducibility of P-450b which is only weakly induced by PB in simple culture media [34].

The mechanism(s) by which dexamethasone modulates different pathways of AAF metabolism are unclear. A broad effect of glucocorticoids on the survival of hepatocytes in primary culture [30, 35–37] may contribute to somewhat higher levels of all pathways for AAF metabolism but cannot account for the differential induction, particularly of 3- and 5-hydroxylation. The inductions of 3-, 5- and *N*-hydroxylations appeared to be maximal (Fig. 2) at dexamethasone concentrations around 30–100 nM, the levels required for maximal glucocorticoid receptor-mediated induction of TAT in comparable cultures (Table 1). At least in the case of 7-hydroxylation, maximal induction apparently required at least micromolar concentrations of dexamethasone consistent with previous reports [3, 9, 10] that induction of P-450p by dexamethasone or PCN may be mediated via a stereospecific mechanism distinct from the classical glucocorticoid receptor. While the glucocorticoids cortisol and corticosterone resembled dexamethasone in their effects on AAF metabolism, some additional non-glucocorticoids including fluoxymesterone and methyltestosterone exerted quite similar effects, suggesting that a range of steroids not restricted to glucocorticoids might act via related mechanisms.

The results in Table 2 suggest that while pregnenolone 16α-carbonitrile induced relatively specific changes in 7-hydroxylation, dexamethasone and other steroids induced several activities. Taken

together with previous findings that glucocorticoids induce ethoxycoumarin *O*-deethylase [19, 32], aldrin epoxidase and arylhydrocarbon hydroxylase [38], bile acid hydroxylation [39], *p*-chloro-*N*-methylalanine demethylase [40], ethylmorphine and aminopyrine *N*-demethylases [8] and also to enhance synthesis of the phenobarbital-inducible P-450 [41] in hepatocyte cultures, it seems apparent that dexamethasone and other steroids have a role in regulating a variety of cytochrome P-450 isozymes. Further the difference in responses to such compounds *in vivo* and in cultured cells suggest that factors yet to be identified strongly influence the steroid-induced pattern of P-450 gene expression.

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