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EFFECT OF NATURAL AND SYNTHETIC GLUCOCORTICOIDS ON RAT HEPATIC MICROSOMAL DRUG METABOLISM

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SUMMARY

Endogenous and synthetic glucocorticoids of varied biological potency have been used for the treatment of intact male rats in an attempt to determine the interactions of corticosteroids with hepatic mixed-function oxidation. The more potent synthetic steroids increased several components of the microsomal electron transport chain, and the metabolism of biphenyl, aniline, benzo[α]pyrene and ethylmorphine. In contrast, the natural glucocorticoids and their less potent synthetic analogues decreased or had no effect on the activity of these parameters. None of the steroids used affected the spectral interaction kinetics or the apparent K_m values of the enzymes responsible for the metabolism of type I and type II substrates. The effects of glucocorticoids on hepatic drug metabolism therefore appear to be different from those of phenobarbital or 3-methylcholanthrene, but show some similarities with those resulting from pregnenolone-16α-carbonitrile or spironolactone pretreatment.

INTRODUCTION

It has been previously shown that the administration of glucocorticoids restores drug metabolism activity in adrenalectomized male rats to normal levels [1–3]. Elevated levels of circulating glucocorticoids, produced in intact rats by the administration of the steroids or by the stimulation of endogenous hormone secretion, result in variable effects on drug metabolism activity. Thus, Bousquet *et al.*[4] demonstrated an increased *in vivo* metabolism of hexobarbital after the injection of ACTH or corticosterone into rats, and Stitzel and Furner[5] found increases in hepatic aniline 4-hydroxylase and ethylmorphine N-demethylase activities by submitting rats to stress. However, rat hepatic aminopyrine N-demethylation has been shown to be inhibited by the administration of corticosterone [6] and a similar effect on hexobarbital oxidation was observed with cortisol [7]. In contrast, some synthetic glucocorticoids have been shown to increase drug metabolizing enzyme activity in liver [8, 9] and extrahepatic tissues [9, 10].

The nature of the changes in the components of mixed-function oxidation resulting from glucocorticoid administration or adrenalectomy are not well defined. Castro *et al.*[11] proposed that adrenalectomy may reduce the activity of the reductases involved in mixed-function oxidation. Ichii and Yago[12] also reported that NADPH-cytochrome c reductase activity was reduced by adrenalectomy and found that the same was true for cytochrome P-450 concentrations. More recently, Hamrick *et al.*[13] observed that the administration of large doses of cortisone acetate to male rats increased NADPH-cytochrome c reductase activity, but decreased cytochrome P-450 concentrations.

In the present study, we have examined the effects of repeated small doses of endogenous and therapeutically important synthetic glucocorticoids on the components of the microsomal electron transport chain and its associated drug metabolizing enzyme activities in rat liver.

MATERIALS AND METHODS

Animals. Male Wistar albino rats aged 38 days at sacrifice were used in all experiments. Control and test groups, each comprising 4 animals, were kept separately in polypropylene cages with stainless steel tops and on Sterolit bedding. All animals were allowed free access to tap water and Spiller's No. 1 small animal diet.

Steroids. Corticosterone (11β,21-dihydroxy-4-pregnene-3,20-dione) was obtained from Sigma, London. Prednisolone (11β,17,21-trihydroxy-1,4-pregnadiene-3,20-dione) was purchased from Koch Light Laboratories, Colnbrook, Bucks. 6β-Hydroxycorticosterone (6β,11β,21-trihydroxy-4-pregnene-3,20-dione), triamcinolone (9α-fluoro-11β,16α,17,21-tetrahydroxy-1,4-pregnadiene-3,20-dione), triamcinolone acetonide (triamcinolone-16α,17α-acetonide), beclomethasone (9α-chloro-16β-methyl-11β,17,21-trihydroxy-1,4-pregnadiene-3,20-dione) and dexamethasone (9α-fluoro-16α-methyl-11β,17,21-trihydroxy-1,4-pregnadiene-3,20-dione) were gifts from various sources.

Substrates. Biphenyl, aniline and aniline hydrochloride were obtained from B.D.H., Poole, Dorset; ethylmorphine was purchased from May and Baker,

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Dagenham, Essex; benzo[α]pyrene and cytochrome c were obtained from Sigma, London, the latter as a ~ 70% pure solid.

Biphenyl was twice recrystallized from ethanol to give a product of m.p. 70°C and aniline was redistilled before use. Other substrates were used without purification.

Animal pretreatment. Solutions of the respective steroids in propylene glycol (3 ml per kg body weight) were injected intraperitoneally once daily for seven consecutive days. In all cases, equimolar amounts of steroid were administered (7.65 μmol/kg/day, equivalent to 2.7 mg/kg/day for corticosterone) and control animals received propylene glycol alone. The last injection was given 16 h before sacrifice, which was always between 08.30 and 09.30.

Tissue preparation. Rats were killed by cervical dislocation and the livers were removed and transferred to ice-cold aq. KCl (1.15% w/v). After removal of adhering blood, fat and connective tissue, livers were blotted dry, weighed and homogenized in 2.5 vol. of 1.15% aq. KCl using a Potter–Elvehjem glass and Teflon homogenizer. The resultant suspension was spun at 10,000 *g* for 20 min at 4°C in an M.S.E. High Speed 18 centrifuge fitted with an 8 × 50 ml rotor. The resulting supernatant was decanted and recentrifuged at 105,000 *g* for 1 h at 4°C (8 × 25 ml rotor in an M.S.E. Superspeed 50 centrifuge). The supernatant was discarded and the remaining microsomal pellet was resuspended in 1.15% aq. KCl as a suspension containing between 4 and 8 mg microsomal protein per ml. Where washed microsomes were required, a further centrifugation at 105,000 *g* was performed prior to resuspension in 0.1 M phosphate buffer (pH 7.4) containing 30% (v/v) glycerol.

Enzyme assays. The NADPH generating system was identical in all the drug metabolism assays performed and contained 1.75 μmol NADP, 15 μmol isocitric acid, 10 μmol $MgSO_4$ and 0.5 units isocitrate dehydrogenase. Enzyme assays were performed largely as originally described by Creaven *et al.*[14] for biphenyl 2- and 4-hydroxylases, by Guarino *et al.*[15] for aniline 4-hydroxylase, by Kuntzman *et al.*[16] for benzpyrene hydroxylase and by Holtzman *et al.*[17] for ethylmorphine N-demethylase. Substrate concentrations used in the assay for biphenyl, aniline, benzo[α]pyrene and ethylmorphine were 3.75, 10.0, 0.1 and 5.0 mM respectively. In studies of the kinetics of metabolism of biphenyl and aniline the concentrations used in the assay were 0.1, 0.133, 0.167, 0.217, 0.313, 0.5 and 1.0 mM for biphenyl and 0.025, 0.033, 0.05, 0.067, 0.111 and 0.25 mM for aniline. Cytochrome b_5 was determined by the method of Klingenberg[18], cytochrome P-450 by that of Omura and Sato[19] and NADPH-cytochrome c reductase by that of Gigon *et al.*[20]. *The minor modifications* used in these, and the drug metabolism assays, have been previously described [21]. Hepatic microsomal protein content was determined by the method of Lowry *et al.*[22].

Binding spectra. The spectrally apparent interactions of biphenyl and aniline with cytochrome P-450 were determined in suspensions of washed microsomes containing 1 mg microsomal protein per ml. Difference spectra were obtained over the range 350 nm to 500 nm by making serial additions of substrate and pure solvent to the sample and reference cuvettes respectively, and by monitoring the relative absorbance changes on a Perkin–Elmer 356 dual beam, dual wavelength recording spectrophotometer. The kinetics of binding of biphenyl and aniline were investigated using concentrations of 0.04, 0.05, 0.06, 0.08, 0.1, 0.14, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8 and 1.0 mM biphenyl, and 0.2, 0.3, 0.4, 0.6, 0.8, 1.2 and 2.0 mM aniline, in the cuvettes.

Calculation of results. The extinction coefficients used in calculations of the microsomal content of cytochromes P-450, b_5 and cytochrome c for NADPH-cytochrome c reductase activity were 91, 185 and 18.5 $mM^{-1} cm^{-1}$ respectively [23, 24]. Statistical evaluation of the results from control and test groups was made using Student's *t*-test. The best fit of a line to a set of points obtained in kinetic experiments was determined using an Olivetti Programma 101 to perform linear regression analysis.

Table 1. Alterations in cytochrome P-450 content, NADPH-cytochrome c reductase activity and several drug metabolizing enzyme activities in rat liver following glucocorticoid administration

Steroid	Cytochrome[1] P-450	NADPH-cytochrome[2] c reductase	Activity Biphenyl 4-[3] hydroxylase	Biphenyl 2-[3] hydroxylase	Aniline 4-[3] hydroxylase	Benzpyrene[3] hydroxylase	Ethylmorphine N-[3] demethylase
Corticosterone	86	89	61*	71	80	79	78
6β-Hydroxycorticosterone	90	80*	75	121	81*	82	69*
Prednisolone	112	109	80	104	103	97	110
Triamcinolone	87		85	79	121	98	132
Triamcinolone acetonide	90	139*	110	125	217*	142*	153
Beclomethasone	117	133*	133	115	168*	141*	142
Dexamethasone	132*	175*	214*	154*	257*	240*	208*

Steroid pretreatment is described in the text. Each value is the mean from 4 animals and is expressed as a percentage of the control.

* Values significantly different ($P < 0.05$) from those in control animals.

[1] Cytochrome P-450 content was determined as nmol per mg microsomal protein.

[2] NADPH-cytochrome c reductase activity was determined as nmol cytochrome c reduced per mg microsomal protein per hour.

[3] Enzyme activities were determined as nmol product formed per mg microsomal protein per hour.

RESULTS

Effects of glucocorticoid pretreatment on hepatic mixed-function oxidation. Table 1 shows the effect of administration of seven different glucocorticoids on cytochrome P-450 content, NADPH-cytochrome c reductase activity and several drug metabolizing enzyme activities in rat liver microsomes. Of all the steroids used for pretreatment, only dexamethasone significantly elevated hepatic microsomal cytochrome P-450 content. However, the converse depression in cytochrome P-450 content appeared to result from pretreatment with corticosterone. While this natural glucocorticoid also tended to depress the activity of NADPH-cytochrome c reductase, pretreatment with triamcinolone acetonide, beclomethasone or dexamethasone resulted in an increased activity of the reductase (Table 1). Cytochrome b_5 levels were largely unaffected by glucocorticoid pretreatment, although triamcinolone, triamcinolone acetonide and dexamethasone decreased the microsomal content of cytochrome b_5 to ~80% of that found in control rats. None of the steroids used significantly affected liver weight (per 100 g body weight) or hepatic microsomal protein content (mg per g liver). However, some of the more potent synthetic glucocorticoids markedly impaired the body growth rate.

Despite the range of effects produced from administration of the various glucocorticoids used, most of the enzyme activities examined responded similarly to a particular steroid, although some quantitative differences emerged. In particular, biphenyl 2-hydroxylase activity was less markedly affected by pretreatment than the other hydroxylating enzymes.

From those glucocorticoids listed in Table 1, only the biologically more potent synthetic steroids increased enzyme activities, while the less potent synthetic and natural corticosteroids tended to have little effect, or produced a depression of enzyme activity. Thus, corticosterone decreased enzyme activities (by 20–39%), prednisolone and triamcinolone induced few changes in activity and triamcinolone acetonide, beclomethasone and dexamethasone increased all the drug metabolizing enzyme activities studied. Dexamethasone was most potent in this respect and increased enzyme activities by 54–157%.

Effect of dexamethasone pretreatment of varying duration on mixed-function oxidation. The magnitude of the changes in enzyme activity resulting from glucocorticoid pretreatment (Table 1) did not appear to correlate quantitatively with any single change in the components of the mixed function oxidase system which were examined. However, it seemed that substantial changes in drug metabolizing enzyme activities could correspond with the combined alterations in the microsomal content of cytochrome P-450 and its rate of reduction (as determined by NADPH-cytochrome c reductase activity). As slow changes in these parameters were suspected over the 7 days of pretreatment, their development was examined after daily administration of dexamethasone for between 1 and 7 days.

The time course of the changes in cytochrome P-450 and cytochrome b_5 content and in NADPH-cytochrome c reductase activity are shown in Fig. 1. Cytochrome P-450 concentrations decreased over the first 5 days of dexamethasone administration, but underwent a rapid increase during the sixth and seventh days of treatment and finally reached values 132% of those in control animals. NADPH-cytochrome c reductase activity was increased at all times after dexamethasone pretreatment. The highest activities were attained after 5 continuous daily doses of dexamethasone and were ~260% of control values. The microsomal content of cytochrome b_5 was, in contrast, decreased after dexamethasone administration, and reached minimum concentrations (60% of control levels) 3 days after the start of treatment.

Figure 2 shows the changes in aniline 4-hydroxylase and biphenyl 2- and 4-hydroxylase activities after

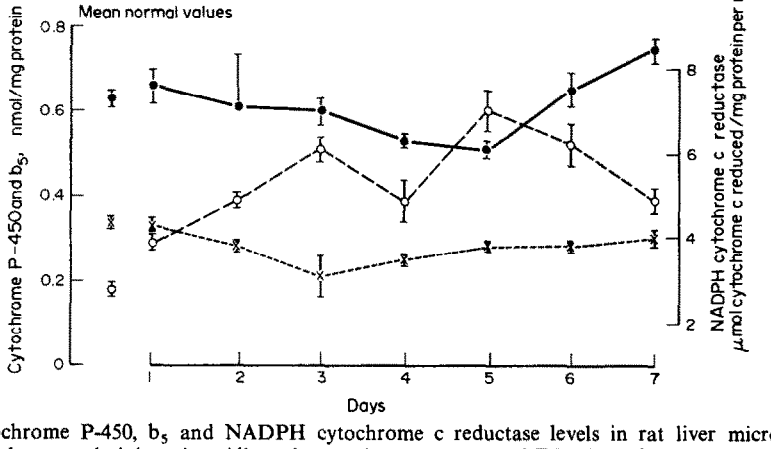

Fig. 1. Cytochrome P-450, b_5 and NADPH cytochrome c reductase levels in rat liver microsomes after dexamethasone administration. All results are given as mean ± S.E.M. from four animals. Control values were determined on days 1, 4 and 7 and the means ± S.E.M. from these twelve determinations were: cytochrome P-450 (●——●): 0.63 ± 0.02 nmol/mg protein; cytochrome b_5 (×----×): 0.34 ± 0.01 nmol/mg protein and NADPH cyt. c reductase (O———O): 2.8 ± 0.1 μmol cytochrome c reduced/mg protein/h.

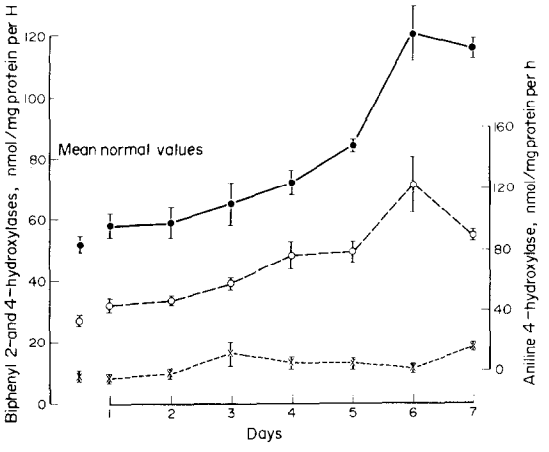

Fig. 2. The alterations in biphenyl and aniline hydroxylation by rat liver microsomes after dexamethasone pretreatment. All results are presented as mean ± S.E.M. from four animals. Control values were determined on days 1, 4 and 7. The means of the determinations (12) on these 3 days were: biphenyl 2-hydroxylase (×——×): 8.2 ± 0.9 nmol/mg protein/h.; biphenyl 4-hydroxylase (●——●): 52 ± 2 nmol/mg protein/h.; aniline 4-hydroxylase (○— —○): 34 ± 2 nmol/mg protein/h.

the dexamethasone pretreatment schedule described in the previous paragraph. Both aniline and biphenyl 4-hydroxylase activities showed similar time dependent responses to dexamethasone administration. Thus, increases in activity were initially slow, but became rapid after 5 days of treatment and reached maximal values thereafter (225% and 300% of control activities for biphenyl and aniline, respectively, after 6 days). In contrast, biphenyl 2-hydroxylase activity responded in a different pattern to repeated dexamethasone administration. After 3 days of treatment, biphenyl 2-hydroxylase activities had risen rapidly to a maximum (200% of control values). Thereafter, activities fell slowly, but remained above control values at all the time points subsequently examined.

Changes in the kinetic constants for the microsomal metabolism and binding of aniline and biphenyl resulting from glucocorticoid pretreatment. In an attempt to elucidate the nature of the changes in enzymic properties resulting from glucocorticoid pretreatment, the kinetics of biphenyl and aniline 4-hydroxylation were examined after 7 days' pretreatment with corticosterone, prednisolone and dexamethasone. Corticosterone and prednisolone had no effect on any of the parameters examined, but dexamethasone significantly increased V_{max} values in the metabolism of both biphenyl and aniline. The K_m values for both hydroxylations were unaffected by dexamethasone pretreatment (Table 2).

Pretreatment of animals with either phenobarbital or the polycyclic hydrocarbon type of inducer exemplified by 3-methylcholanthrene, can affect the binding of type I and type II substrates, and their subsequent metabolism [25, 26]. In order to determine whether similar changes in binding characteristics could result from glucocorticoid pretreatment and whether these changes might influence drug metabolism, the binding of biphenyl (type I) and aniline (type II) to cytochrome P-450 was studied after pretreatment of rats for 7 days with corticosterone, prednisolone and dexamethasone. Interpretation of the curvilinear Lineweaver Burk plot which resulted using biphenyl in these studies has been discussed elsewhere [27]. Prednisolone and corticosterone had no effect on any of the parameters studied. Furthermore, following dexamethasone pretreatment (Table 2) the only change observed was a marginal increase in ΔE_{max} of the low affinity interactions of biphenyl with hepatic microsomes.

DISCUSSION

The present study shows that pretreatment of male rats with small doses of glucocorticoids for 7 days can affect hepatic drug metabolism. The changes produced vary according to the steroid used and the microsomal enzyme activity measured. When increases in activity of the hepatic microsomal mixed-function oxidase system were observed, they were considerably smaller in magnitude than those previously reported with the classical enzyme inducers [28].

Corticosterone and 6β-hydroxycorticosterone (the two endogenous glucocorticoids used in the present study) produced a general depression of the microsomal enzyme activities similar to that observed by Radzialowski and Bousquet[6]. Prednisolone, like triamcinolone, produced no significant changes in any of the parameters examined. Beclomethasone, triamcinolone acetonide and dexamethasone, on the other

Table 2. The effect of dexamethasone pretreatment on the kinetic constants associated with the binding and 4-hydroxylation of aniline and biphenyl in rat liver microsomes

Kinetic constants	Biphenyl		Aniline	
	Control	Treated	Control	Treated
K_m	1.4 ± 0.4	1.1 ± 0.2	0.8 ± 0.1	1.0 ± 0.1
V_{max}	4.1 ± 1.3	11.7 ± 2.3*	8.4 ± 1.3	23.2 ± 3.1*
K_s^1	0.50 ± 0.11	0.41 ± 0.02	5.9 ± 0.5	7.8 ± 0.5
E_{max}^1	1.5 ± 0.3	1.7 ± 0.1	1.4 ± 0.2	1.5 ± 0.2
K_s^2	1.3 ± 0.1	1.3 ± 0.1		
E_{max}^2	2.1 ± 0.1	2.6 ± 0.1*		

All kinetic constants are apparent. K_m and K_s values are 10^{-4} M; V_{max} values are nmol product/mg microsomal protein/h; E_{max} values are changes in extinction × $10^{-2}/2$ nmol cytochrome P-450. The superscripts ^{1} and ^{2} for K_s and E_{max} refer to the binding of biphenyl, and denote values for the high affinity and low affinity interactions, respectively (see text). * results significantly different ($P < 0.05$) from the corresponding control value. Steroid pretreatment is described in the text.

hand, increased microsomal drug metabolizing enzyme activity. Among these steroids, dexamethasone was found to be the most potent in this respect and it alone significantly raised all the activities measured. In view of the long-term therapeutic uses of synthetic corticosteroids, the increases in enzyme activities demonstrable only after several days of dexamethasone pretreatment need further examination over an extended period.

The investigation of the time course of the action of dexamethasone (Fig. 1) showed that the pattern of changes in the drug metabolizing enzymes did not temporally correspond with that of microsomal cytochrome P-450 content or NADPH-cytochrome c reductase activity. However, the results presented in Table 1 and in Figs. 1 and 2 indicate that substantial increases in NADPH-cytochrome c reductase activity resulting from glucocorticoid pretreatment usually corresponded with an elevated rate of drug metabolism, as did a significant increase in cytochrome P-450 concentration. In contrast, decreased concentrations of cytochrome b_5 had little effect on drug metabolizing enzyme activity. These observations are in agreement with those of Ichii and Yago[12] and suggest that glucocorticoid-induced changes in drug metabolizing enzyme activity may be more associated with corresponding changes in cytochrome P-450 content and NADPH-cytochrome c reductase activity than with other components of the microsomal electron transport chain.

Similar to the findings of Hamrick *et al.* [13] with cortisone acetate, we observed that the administration of corticosterone, prednisolone or dexamethasone, unlike phenobarbital or 3-methylcholanthrene [25, 26], produced no appreciable alteration in the binding of type I and type II substrates to male rat liver microsomes. Thus, while phenobarbital and 3-methylcholanthrene induces similar increases in both the biphenyl type I low affinity and high affinity interaction (ΔE_{max}) [27], only the former parameter was affected marginally by dexamethasone. The implication of this phenomenon in biphenyl 4-hydroxylation still remains unknown. In addition, dexamethasone pretreatment induced no changes in the ratio of the 430:355 nm peak heights of the microsomal ethyl isocyanide difference spectrum, nor in the position of the 450 nm absorbance maximum of the dithionite reduced versus CO-dithionite reduced difference spectrum. The apparent K_m values for microsomal biphenyl 4-hydroxylation and aniline 4-hydroxylation were also unchanged by dexamethasone pretreatment, although large increases in the V_{max} of both enzyme reactions were noted.

It can be concluded from our findings that, in the rat, administration of synthetic glucocorticoids does not influence the characteristics of the hepatic microsomal mixed-function oxidase system concerning its interaction with exogenous substrates. However, the enhancement of enzyme activity noted after pretreatment of rats with some of these steroids seems to result from raised V_{max} values which may be related to the faster rates of cytochrome P-450 reduction as indicated by increased NADPH-cytochrome c reductase activity [29]. In this regard, any possible role of glucocorticoid-mediated hepatic enzyme protein synthesis [30] cannot be ascertained until further experiments are performed with known inhibitors of protein synthesis.

In order to explain the differences in the effects of various corticosteroids on drug metabolizing enzyme activities, some facets of their possible action need consideration. The administration of glucocorticoids would, *via* the suppression of the hypothalamic-pituitary-adrenal axis (HPA), cause a diminution of the secretion of endogenous corticosteroids and their continuous effect. In its place would be substituted the resultant effect of a sudden upsurge to a high level of the administered steroid, the qualitative and quantitative nature of whose action on the microsomal enzymes would be largely determined by its interaction with the cellular components. One of the factors that would regulate the duration and magnitude of corticosteroid action on both the HPA axis and on the liver is its rate of metabolism. Whether this may be responsible for the differences between various steroids observed in the present study must await more detailed knowledge of the metabolism of synthetic corticosteroids in the rat.

Thus, the present study suggests that the induction characteristics of dexamethasone are different from those of both phenobarbital and 3-methylcholanthrene [31]. However, dexamethasone shows some resemblance to pregnenolone-16α-carbonitrile in that both compounds increase the microsomal content of cytochrome P-450 and NADPH-cytochrome c reductase activity [29]. In contrast, prednisolone and triamcinolone affect neither the cytochrome nor the reductase, whereas beclomethasone and triamcinolone acetonide, like the aldosterone antagonist steroid spironolactone, increased only NADPH-cytochrome c reductase activity [32].

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