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SHORT COMMUNICATIONS

Dexamethasone induces hepatic cytochrome P-450 content and increases certain monooxygenase activities in rhesus monkey fetuses

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Cytochrome P-450s are present only at very low concentrations in fetal liver from rats and other common laboratory mammals. In such species, total cytochrome P-450 content begins to increase at birth [1–3]. At least one constituent form of the cytochrome develops postnatally in untreated rat pups, but additional sex-dependent forms develop at puberty to give rise to the characteristic sex-differences in monooxygenase activity seen in adult rats [4].

We have shown previously that at least one form of hepatic cytochrome P-450 and several monooxygenase activities, including aminopyrine-*N*-demethylase and 7-ethoxycoumarin-*O*-deethylase, are increased in neonatal and sexually immature rats following treatment with glucocorticoid hormones such as dexamethasone or corticosterone [5, 6]. Moreover, adrenalectomy of neonatal rat pups inhibits the subsequent development of hepatic cytochrome P-450 [5].

Glucocorticoid treatment does not, however, increase hepatic cytochrome P-450 concentration or monooxygenase activity when given prenatally in the rat. In common with many other neonatally-developing rat liver enzymes, fetal liver cytochrome P-450(s) are not competent to respond to glucocorticoid treatment *in utero* in this species [2, 3]. This lack of competence is not due to failure of the glucocorticoids to reach the fetal liver, because treatment with these steroids readily increases activities of other fetal liver enzymes, such as the "late fetal" form of UDP-glucuronyltransferase [7]. Evidence suggests [2, 8–10] that repressive hormonal factors may inhibit the response of the neonatally developing enzymes to glucocorticoids until after birth, but that enzymes such as the "late fetal" form of UDP-glucuronyltransferase are not affected by these factors and so develop during late gestation in response to the increasing corticosterone concentrations in the fetal plasma [3].

Primate liver cytochrome P-450(s) show different developmental profiles to those of the rat. In both human and monkey fetuses, significant amounts of hepatic cytochrome P-450(s) are present throughout mid and late gestation [11, 12]. Some monooxygenase activities are also present (e.g. aminopyrine-*N*-demethylase), but others appear to be low or absent (e.g. benz(a)pyrine hydroxylase) [11].

Presence of hepatic cytochrome P-450(s) in the primate fetus could imply that some form(s) of the cytochrome are already competent to respond to glucocorticoid *in utero*, and we have, therefore, in this study, investigated the effects of glucocorticoid treatment on the cytochrome P-450-dependent monooxygenase system in rhesus monkey fetuses.

Methods

Fetuses from time-mated rhesus monkeys *(Macaca mulatta)* were used at gestational ages between 142 and 146 days. Pregnant animals received 10 mg dexamethasone in sesame oil per kg body wt by s.c. injection 3, 2 and 1 days before surgery. Collection of fetal livers and liver biopsy samples from non-pregnant adult female monkeys has been described elsewhere [13].

Time-mated, pregnant Wistar rats were injected (s.c.) with 10 mg of dexamethasone in sesame oil per kg body wt on days 17, 18 and 19 of gestation and sacrificed on day 20. Control rats received injection of sesame oil alone. The fetuses were removed, decapitated and their livers pooled so that a single sample (8–16 livers) was obtained for each pregnant rat. Central lobes of livers from adult female rats (50–70 days old) were also used. Microsomal suspensions were prepared in duplicate from the monkey liver samples or singly from the rat liver samples as described previously [13]. The washed microsomal pellets were resuspended in 0.1 M potassium phosphate buffer pH 7.4 containing 20% glycerol and stored at 0–5°C until assay, within 48 hr. No significant loss of cytochrome P-450 or monooxygenase activity occured during this time period under these conditions.

Cytochrome P-450 reductase and aminopyrine-*N*-demethylase activities and cytochrome P-450 content were assayed by the methods of Phillips and Langdon [14], Matsubara *et al.* [15], and Omura and Sato [16] respectively; 7-ethoxycoumarin-*O*-deethylase and 7-ethoxyresorufin-*O*-deethylase were assayed by the methods of Prough *et al.* [17]. Microsomal protein content was measured by the method of Lowry *et al.* [18].

Results and discussion

As expected, microsomal cytochrome P-450, its reductase and aminopyrine-*N*-demethylase activities were all present in significant amounts in fetal rhesus monkey liver (Table 1) when compared with their content and activities in fetal rat liver (Table 2). However, the microsomal suspensions prepared from the adult female monkeys contained relatively high concentrations of cytochrome P-450 and aminopyrine-*N*-demethylase activities so that the amounts present in the fetal monkey liver represent only 17% and 12% of adult values respectively. Microsomal 7-ethoxycoumarin-*O*-deethylase activity, though present in the monkey fetal liver microsomal suspensions, was only at 4% of adult values, whereas hepatic 7-ethoxyresorufin-*O*-deethylase activity was absent from the monkey fetuses, but was present in the adult monkey liver (Table 1).

Maternal dexamethasone treatment increased microsomal cytochrome P-450 content and cytochrome P-450 reductase, aminopyrine-*N*-demethylase and 7-ethoxycoumarin-*O*-deethylase activities (but not 7-ethoxyresorufin-*O*-deethylase activity) in the fetal monkey liver when compared to untreated control values (Table 1). The control monkey fetuses used in these experiments were from untreated, rather than vehicle-treated, mothers because we have previously shown that the injection procedure itself can increase endogenous glucocorticoid concentrations in the fetal serum [19] and thus possibly influence the development of fetal liver enzymes. However, in an additional experiment, a single pregnant monkey was injected with sesame oil alone, analogously to the dexamethasone-treated monkeys. The resulting microsomal cytochrome P-450 content, cytochrome P-450 reductase activity and aminopyrine-*N*-demethylase activities in the fetal liver were within the S.E.M. ranges of their

Table 1. Effect of dexamethasone pretreatment on hepatic microsomal cytochrome P-450 content and monooxygenase activities in rhesus monkey fetuses

Enzyme activity	Fetal liver (142–145 days) Untreated (3)	Dexamethasone-treated (3)	% of control	Adult liver untreated (3)
Cytochrome P-450 (nmol per mg of protein)	0.23 ± 0.03	0.40 ± 0.03	174 $P < 0.01$	1.37 ± 0.04
Cytochrome P-450 reductase (nmol/min per mg of protein)	107 ± 12	162 ± 9	151 $P < 0.02$	220 ± 27
Aminopyrine-*N*-demethylase (nmol/min per mg of protein)	1.34 ± 0.04	2.87 ± 0.18	213 $P < 0.02$	11.6 ± 1.0
7-Ethoxycoumarin-*O*-deethylase (pmol/min per mg of protein)	31 ± 3	156 ± 46	503 $P < 0.05$	891 ± 88
7-Ethoxyresorufin-*O*-deethylase (pmol/min per mg of protein)	<1.0	<1.0	—	33 ± 12

Pregnant rhesus monkeys either received 10 mg dexamethasone in sesame oil per kg body wt, 3, 2 and 1 days before assay, or were untreated. The fetuses were sacrificed at 142–146 days of gestation and liver microsomal suspensions prepared as outlined in the Methods section. The adult monkeys used were 10-year-old females. Liver microsomal suspensions were prepared from biopsy samples taken from their central hepatic lobes. Results are expressed as mean ± S.E.M. Sample numbers (in parentheses) refer to the number of animals used.

Table 2. Effect of dexamethasone pretreatment on hepatic microsomal cytochrome P-450 content and monooxygenase activities in rat fetuses

Enzyme activity	Fetal liver (20 days gestation) Control (4)	Dexamethasone-treated (3)	% of control	Adult liver untreated (3)
Cytochrome P-450 (nmol per mg of protein)	0.062 ± 0.016	0.063 ± 0.007	102 NS	0.737 ± 0.079
Cytochrome P-450 reductase (nmol/min per mg of protein)	37.0 ± 7.4	66.8 ± 8.1	180 $P < 0.05$	170.2 ± 17.1
Aminopyrine-*N*-demethylase (nmol/min per mg of protein)	0.010 ± 0.008	0.015 ± 0.010	150 NS	2.72 ± 0.25
7-Ethoxycoumarin-*O*-deethylase (pmol/min per mg of protein)	1.6 ± 0.2	1.4 ± 0.4	88 NS	163 ± 30
7-Ethoxyresorufin-*O*-deethylase (pmol/min per mg of protein)	<1.0	<1.0	—	299 ± 55

Pregnant rats received either 10 mg dexamethasone in sesame oil per kg body wt or sesame oil alone on the 17th, 18th and 19th days of gestation. The fetuses were sacrificed on Day 20 and liver microsomal suspensions prepared as outlined in the Methods section. The adult rats used were 50- to 70-day-old females. Results are expressed as in Table 1 except that the sample number for the fetuses refers to the number of pooled litters used (NS = not significant).

respective values for the fetal liver from the untreated monkeys. This suggests that the dexamethasone rather than the sesame oil vehicle is responsible for the increases in cytochrome P-450 and monooxygenase activity.

Apparent induction of cytochrome P-450 and monooxygenase activity in fetal liver following phenobarbital treatment has been attributed to artifactual differences in the recovery of the microsomal fractions [20]. Aminopyrine-*N*-demethylase activity was therefore routinely assayed in the 8000 *g* pellet fraction in addition to the 100,000 *g* microsomal fraction to determine whether dexamethasone caused similar differences in microsomal recovery. Dexamethasone treatment increased the aminopyrine-*N*-demethylase activity in the fetal monkey liver 8000 *g* pellet fractions 4-fold, from 0.08 ± 0.03 (3) to 0.34 ± 0.04 (3) nmol min^{-1} per mg of protein ($P < 0.01$), suggesting that total hepatic aminopyrine-*N*-demethylase activity is increased following dexamethasone treatment.

The induction of cytochrome P-450 and monooxygenase activity in fetal monkey liver following dexamethasone treatment contrasts with the lack of response of this liver enzyme system to analogous dexamethasone treatment in the fetal rat (Table 2). Microsomal cytochrome P-450 content remains at 8–10% of adult (female) values although there is some stimulation of the reductase. Neither aminopyrine-*N*-demethylase, 7-ethoxycoumarin-*O*-deethylase nor 7-ethoxyresorufin-*O*-deethylase activities are significantly increased by the dexamethasone treatment in this species. All three activities, however, can be increased at least 2-fold in liver from 10-day old pups, following analogous dexamethasone treatment (J. E. A. Leakey, unpublished results).

Dexamethasone therapy has been used therapeutically to stimulate lung surfactant synthesis in premature human neonates [21] and has been shown to stimulate the development of hepatic UDP-glucuronyltransferase activity towards bilirubin in the rhesus monkey fetus [19]. We have demonstrated here that such therapy may also induce at least one form of hepatic cytochrome P-450 in the primate fetus. However, if similar cytochrome P-450 isozyme substrate specificities occur in the rat and the monkey, the lack of response of hepatic 7-ethoxyresorufin-*O*-deethylase activity to dexamethasone induction in the monkey fetus would suggest that glucocorticoid therapy might not necessarily increase the perinatal primates' susceptibility to metabolically activable carcinogens.

In summary, we have demonstrated that in the developing primate, hepatic cytochrome P-450 content, aminopyrine-*N*-demethylase and 7-ethoxycoumarin-*O*-deethylase activities are all readily inducible by dexamethasone in

the rhesus monkey fetus during late gestation whereas they are not inducible by this glucocorticoid in fetal rat liver. Hepatic 7-ethoxyresorufin-*O*-deethylase activity is not inducible by dexamethasone in the fetal monkey even though this monooxygenase activity is present in adult monkey liver and is inducible by dexamethasone in postnatal rat liver.

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Induction of rat liver microsomal cytochrome P-450 by muscone (3-methylcyclopentadecanone)

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Muscone (3-methylcyclopentadecanone) is the ordorous principle of musk, a product usually made from the musk glands of male musk deer. Musk is a component in many perfumes and is considered to be a precious ingredient in traditional Chinese medicine. Although musk has been used for thousands of years for the treatment of various diseases and malfunctions, its biological activities have not been characterized. It has been reported that pretreatment of rats with musk or muscone increases the clearance of pentobarbital and shortens pentobarbital-induced sleeping time [1]. Such effects have also been observed with mice (X. Zhu, unpublished observations). It has been suggested that the effects are due to the induction of liver microsomal enzymes [1]. However, direct evidence for such induction was not available. In this communication, we report the induction of rat liver microsomal cytochrome P-450 by muscone.

Materials and methods

Chemicals. Isocitrate dehydrogenase, DL-isocitric acid, NADP, NADPH, and metyrapone were obtained from the Sigma Chemical Co. (St. Louis, MO). *p*-Nitroanisole was from the Eastman Organic Co. (Rochester, NY). *N*-Nitrosodimethylamine was purchased from the Aldrich Chemical Co. (Milwaukee, WI). Chemicals received as gifts were: chemically synthesized muscone from the Shangdong Pharmaceutical Co. (Jinan, Shandong, China), ethylmorphine-HCl from Merck & Co. (Rahway, NJ), and benzphetamine-HCl from the Upjohn Co. (Kalamazoo, MI).

Animals and microsomes. Male Sprague–Dawley rats (body weight 50–65 g) were obtained from Taconic Farms, Germantown, NY. They were fed a commercial laboratory chow (Ralston Purina Co., St. Louis, MO) and water *ad lib.* Muscone was dissolved in corn oil and administered by a single intraperitoneal injection 22–24 hr before the animals were killed. The animals in the control group received the vehicle only. In other induction studies, the rats received a daily intraperitoneal injection of phenobarbital (75 mg/kg in saline) or 3-methylcholanthrene (25 mg/kg in corn oil) for 3 days. Liver microsomes were prepared by differential centrifugation and washed once with a solution containing 154 mM KCl and 10 mM EDTA as described previously [2]. The microsomal samples were