Dexamethasone increases UDP-glucuronyltransferase activity towards bilirubin, oestradiol and testosterone in foetal liver from rhesus monkey during late gestation

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We previously showed that in perinatal rhesus monkeys hepatic UDP-glucuronyltransferase activities appear to develop differentially in two clusters, analogous to those of the rat. We demonstrate here that hepatic UDP-glucuronyltransferase activities differ between the rat and the rhesus monkey in their response to glucocorticoids. Treatment of pregnant rhesus monkeys with dexamethasone during late gestation increases UDP-glucuronyltransferase activities towards bilirubin, oestradiol and testosterone in the foetal-liver microsomal fraction to 25, 4 and 4 times their low control values respectively. Analogous dexamethasone treatment in rat fails to increase these activities significantly in the foetal liver. These findings suggest that maternal glucocorticoid therapy in late gestation could greatly increase the newborn primate's capacity to glucuronidate bilirubin.

UDP-glucuronyltransferase (EC 2.4.1.17) is absent from early foetal liver, but activities towards many of its endogenous and xenobiotic substrates develop perinatally in most species, including man, where delayed development of the transferase activity towards bilirubin may contribute to postnatal jaundice (see Dutton & Leakey, 1981; Leakey, 1983). It is now well established that in rat liver UDP-glucuronyltransferase activities develop in two clusters (as defined by Greengard, 1971), which differ in substrate specificity. Activities belonging to cluster 1 surge towards adult values during late gestation, whereas those belonging to cluster 2 remain low until birth and then rise neonatally (Wishart, 1978).

Evidence suggests that the development of cluster-1 activities is triggered by corticosterone (Wishart & Dutton, 1977), which also surges in its concentration in the foetal plasma during late gestation in the rat (Martin *et al.*, 1977). The mechanism controlling the development of cluster-2 activities is less well understood. These activities are not increased in foetal rat liver by either endogenous corticosterone or glucocorticoid therapy, but, as with many other enzymes that develop neonatally, they become competent to respond to glucocorticoid stimulation at birth,

and subsequent dexamethasone treatment in neonatal rats does increase UDP-glucuronyltransferase activity towards several substrates which develop in cluster 2 (Leakey, 1983, 1984). It is unclear why these activities do not respond to glucocorticoid *in utero*, but it has been suggested that high foetal concentrations of potentially repressive hormones may be responsible (Dutton & Leakey, 1981).

We have previously shown that, in the developing rhesus monkey (*Macaca mulatta*), hepatic UDP-glucuronyltransferase activities also appear to develop differentially, in two clusters which are analogous to those of the rat. UDP-glucuronyltransferase activities towards 2-aminophenol, 5-hydroxytryptamine, 1-naphthol and 4-nitrophenol (cluster 1 in the rat) were all present at near-adult values in liver from rhesus-monkey foetuses during late gestation, whereas activities towards bilirubin, oestradiol and testosterone (cluster 2 in the rat) were only present at less than 5% of adult values (Leakey *et al.*, 1983).

There are however, differences between the rat and the rhesus monkey in other aspects of foetal liver development. For instance, there are significant amounts of smooth endoplasmic reticulum and microsomal cytochrome *P*-450 present in

foetal liver during late gestation in most species of primate (Nau & Neubert, 1978), whereas both of these develop in cluster 2 in the rat (Leakey, 1983). These differences imply that there may also be differences between the rat and the rhesus monkey in the mechanisms controlling perinatal development of liver enzymes, and we have therefore investigated the effects of glucocorticoids on the cluster-2 activities of UDP-glucuronyltransferase in the rhesus monkey.

In the present paper, we report that UDP-glucuronyltransferase activities towards bilirubin, oestradiol and testosterone are at least partially competent to respond to dexamethasone stimulation in foetal liver from rhesus monkeys, but not in that from rats.

Experimental

Foetuses from time-mated rhesus monkeys (*Macaca mulatta*) were used at gestational ages between 142 and 146 days. Term in this species is 165 ± 5 days. Pregnant animals received 10 mg of dexamethasone/kg body wt. in sesame oil by subcutaneous injection 3, 2 and 1 days before surgery. The foetuses were delivered by Caesarian section, and foetal liver microsomal suspensions prepared as described previously (Leakey *et al.*, 1983), except that in some cases portions of the initial homogenates were also kept and assayed. The microsomal suspensions could be stored for up to 48 h at $0-4^{\circ}\text{C}$ or for longer periods at -20°C without significant loss of activity when assayed under optimally activating conditions (Leakey *et al.*, 1983). This allowed sufficient time to complete the assays even after, in some cases, transport of the samples from Arkansas to Scotland by air.

Time-mated pregnant Wistar rats were injected (intraperitoneally) with 10 mg of dexamethasone in sesame oil/kg body wt. on days 17, 18 and 19 of gestation and killed on day 20. The foetuses were removed, decapitated and trunk blood samples collected. Their livers were pooled so that a single sample (8–16 livers) was obtained from each pregnant rat. Microsomal suspensions were prepared from these pools by the methods used for the monkey liver.

Endogenous glucocorticoid concentrations were measured in serum samples of blood taken from the foetal monkeys during exsanguination, or foetal rat trunk blood, with the use of radioimmunoassay method described by Poland *et al.* (1981). The anti-glucocorticoid serum, which was specific and equally sensitive for corticosterone and cortisol, but was inactive against dexamethasone, was obtained from Radioassay Systems Laboratories, Carson, CA, U.S.A. (catalogue no. 1470). The [^3H]corticosterone used in the assay was

obtained from Amersham International, Amersham, Bucks., U.K., and purified by h.p.l.c. by the method of Shimizu *et al.* (1983).

Microsomal glycogen concentration was measured by the method of Van Handel (1965), whereas protein concentrations and optimally activated UDP-glucuronyltransferase activities were assayed as described previously (Leakey *et al.*, 1983).

Bilirubin conjugates were analysed by the methods of Blanckaert *et al.* (1980) as follows; 75 μl samples of the microsomal suspensions were incubated with 0.35 mM-bilirubin, 6 mM- MgCl_2 , 0.3% digitonin, 4% bovine serum albumin, 4 mM-UDP-glucuronic acid, and 50 mM-Tris/HCl buffer, pH 7.65, in a final volume of 250 μl for 45 min in the dark at 37°C . Control incubations contained distilled water instead of UDP-glucuronic acid. After incubation, 100 μl of 300 mM-potassium phosphate buffer, pH 6.8, was added to each tube and the resulting mixtures were extracted with 2×1 ml of chloroform to remove excess unconjugated bilirubin. After this, 250 μl samples of the aqueous phase were then subjected to alkaline methanolysis by the method of Blanckaert (1980), except that the reaction mixture was scaled down by a factor of 2. The methyl bilirubin derivatives of the bilirubin conjugates were finally dissolved in 250 μl of chloroform, and 40–50 μl samples were analysed on an Altex 110A h.p.l.c. unit fitted with a Li-Chrosorb Si 60 5 μ column (HPLC Technology, Macclesfield, Cheshire, U.K.).

In some cases, the 300 mM-potassium phosphate buffer was replaced by a 250 mg/ml suspension of bacterial β -glucuronidase (Sigma Chemical Co.; type IX) in either the same phosphate buffer alone or the same buffer with 30 mM-glucaro-1,4-lactone added. In these cases, the resulting incubation mixtures were incubated for a further 15 min specifically to hydrolyse the bilirubin glucuronide conjugates. The retention times of the mono- and di-methyl bilirubin isomers on this h.p.l.c. system were obtained by using the standard compounds kindly provided by Dr. K. P. M. Heirwegh (Leuven, Belgium).

Results

Dexamethasone treatment produced no visible congenital malformations in either the rat or rhesus-monkey foetuses, but in both species the foetuses of treated mothers were slightly smaller than those of controls. Dexamethasone treatment increased liver wet weights in the monkey foetuses, but not in the rat foetuses (Table 1). This increase resulted in microsomal fractions with lower recoveries than in those from control monkey foetuses, but which had larger amounts of glycogen sedi-

Table 1. *Effect of dexamethasone pretreatment on foetal liver weight, microsomal protein and glycogen contents and serum glucocorticoid concentration*

Pregnant rats and rhesus monkeys received injections of 10 mg of dexamethasone/kg in sesame oil 3, 2 and 1 days before assay. Control rats received equivalent volumes of sesame oil alone, whereas the control monkeys were untreated. The rat and rhesus-monkey foetuses were killed at 20 and 142–146 days gestation respectively, and blood samples were taken. The livers were removed and weighed, and microsomal suspensions were prepared from either foetal-rat livers pooled from individual litters or duplicate samples of foetal-monkey liver. Microsomal protein and glycogen contents and serum endogenous glucocorticoid (cortisol + corticosterone) were assayed as outlined in the Experimental section. Results are expressed as means $\pm$ S.E.M. The sample number (in parentheses) refers to the number of litters of rat foetuses or the number of monkey foetuses. Statistical significance (* $P < 0.01$, † $P < 0.002$, ‡ $P < 0.001$) was calculated with Student's unpaired t test.

Species . . .	Rat		Rhesus monkey	
	Control	Treated	Control	Treated
Liver wet wt. (g/foetus)	0.019 $\pm$ 0.003 (4)	0.012 $\pm$ 0.003 (4)	9.5 $\pm$ 0.5 (3)	23.1 $\pm$ 2.6* (3)
Microsomal protein (mg/g of liver)	8.0 $\pm$ 1.5 (4)	9.7 $\pm$ 2.0 (4)	11.3 $\pm$ 0.3 (3)	6.9 $\pm$ 0.5† (3)
Microsomal glycogen (mg/g of liver)	42.4 $\pm$ 2.6 (3)	48.7 $\pm$ 3.6 (3)	12.4 $\pm$ 1.3 (3)	42.8 $\pm$ 2.8‡ (3)
Endogenous glucocorticoid (μ g/100 ml of serum)	31.7 $\pm$ 2.4 (4)	4.3 $\pm$ 2.2‡ (3)	6.8 $\pm$ 2.7 (3)	0.9 (1)

menting with them (Table 1). Microsomal glycogen concentration was already high in liver from the control rat foetuses, but was increased further by dexamethasone treatment.

As expected, dexamethasone treatment depressed endogenous glucocorticoid production in the rat foetuses, so that serum concentrations of cortisol plus corticosterone were much lower in serum samples from treated foetuses than from controls. The single result available implied that this occurred in the monkey foetuses also. However, the serum glucocorticoid concentration in control rat foetuses was over 4 times greater than those of the control monkey foetuses ($P < 0.001$). In an initial experiment, a pregnant rhesus monkey (139 days gestation) was pretreated with sesame oil without dexamethasone to determine whether the injection procedure alone was sufficiently stressful to increase the foetal serum glucocorticoid concentration by itself. The resulting endogenous glucocorticoid concentration after 3 days of treatment was 18.8 μ g of corticosterone equivalent/100 ml of serum, or 275% of untreated control values. Because of this, we used untreated control monkeys for these experiments. Injection of sesame oil did not increase serum glucocorticoid concentrations over those of untreated controls in the rat foetuses, possibly because serum corticosterone concentrations are already high in late gestation. The control pregnant rats therefore received injections of sesame oil.

As expected, maternal dexamethasone treatment increased UDP-glucuronyltransferase activities belonging to cluster 1 in foetal liver micro-

somal fraction from both rats and rhesus monkeys. For instance, UDP-glucuronyltransferase activity (mean $\pm$ S.E.M.) towards 5-hydroxytryptamine was increased from 5.96 $\pm$ 1.16 (4) to 9.32 $\pm$ 0.48 (4) ($P < 0.05$) and 0.14 $\pm$ 0.02 (3) to 0.42 $\pm$ 0.13 (3) ($P < 0.05$) nmol/min per mg of protein in the rat and monkey respectively, and that towards 1-naphthol from 8.9 $\pm$ 0.8 (2) to 25.6 $\pm$ 3.1 (2) and from 12.9 $\pm$ 1.8 (3) to 22.1 $\pm$ 2.0 (3) ($P < 0.05$) nmol/min per mg of protein in the rat and monkey respectively.

Table 2 compares the effects of dexamethasone pretreatment on hepatic microsomal UDP-glucuronyltransferase activities belonging to cluster 2 between rat and rhesus-monkey foetuses during late gestation. Dexamethasone did not significantly alter hepatic microsomal UDP-glucuronyltransferase activities towards bilirubin, oestradiol and testosterone in the rat foetuses, nor was there any difference in these activities between sesame-oil-treated and untreated controls. All these activities, however, were significantly increased by dexamethasone treatment in the rhesus-monkey foetuses and, for activity towards bilirubin, the glucocorticoid produced a 25-fold increase.

Such a large increase in the latter activity of UDP-glucuronyltransferase was unlikely to be due to increased recovery of the activity in the microsomal fraction, but, in a single experiment to investigate this possibility, we compared UDP-glucuronyltransferase activity towards bilirubin in the initial homogenates of liver from a control monkey foetus with that from a treated foetus, in addition to assaying the microsomal fractions.

Table 2. *Effect of dexamethasone pretreatment on microsomal UDP-glucuronyltransferase activities*
 The microsomal suspensions, prepared as in Table 1, were assayed for UDP-glucuronyltransferase activities towards bilirubin, oestradiol and testosterone as outlined in the Experimental section. The specific activities (means $\pm$ S.E.M.) are those optimally activated with digitonin (bilirubin) or Brij-58 (steroids). (NS, not statistically different from controls.)

Species	Treatment	UDP-glucuronyltransferase activity (nmol/min per mg of protein)		
		Bilirubin	Oestradiol	Testosterone
Rat (4)	Sesame oil (control)	0.010 $\pm$ 0.002	0.091 $\pm$ 0.020	0.103 $\pm$ 0.010
	Dexamethasone (as % of control)	0.011 $\pm$ 0.002 110 (NS)	0.095 $\pm$ 0.021 104 (NS)	0.084 $\pm$ 0.013 82 (NS)
Monkey (3)	Untreated (control)	0.003 $\pm$ 0.001	0.083 $\pm$ 0.007	0.094 $\pm$ 0.026
	Dexamethasone (as % of control)	0.074 $\pm$ 0.012 2467 ($P < 0.01$)	0.329 $\pm$ 0.058 396 ($P < 0.02$)	0.404 $\pm$ 0.074 430 ($P < 0.02$)

This transferase activity was below the level of accurate detection in both the homogenate (<0.002 nmol/min per mg of protein) and the 8000g pellet fraction (<0.001 nmol/min per mg of protein) prepared from liver from the control foetus, whereas the specific activity of the liver homogenate from the dexamethasone-treated foetus was 0.006 nmol/min per mg of protein. Total liver UDP-glucuronyltransferase activity towards bilirubin is therefore increased by dexamethasone in addition to activity recovered from the microsomal fraction.

The UDP-glucuronyltransferase activity towards bilirubin in the microsomal preparations was investigated further by the techniques of Blanckaert *et al.* (1980), as described in the Experimental section. Fig. 1 shows the h.p.l.c. recorder traces from an assay using liver microsomal fraction from a foetus of a dexamethasone-treated monkey. The presence of UDP-glucuronic acid in the incubation mixture causes the appearance of two small peaks with retention factors corresponding to those of the C-8 and C-12 monomethyl bilirubin isomer mixtures, but dimethyl bilirubin was not detected. The monomethyl bilirubin peaks were not obtained after methanolysis of UDP-glucuronic acid-containing incubation mixtures additionally incubated with β -glucuronidase, unless glucaro-1,4-lactone was also included in the second incubation. Furthermore, with the use of hepatic microsomal fractions from control monkey foetuses, no methylbilirubins were produced from UDP-glucuronic acid-supplemented incubation mixtures.

Treatment with dexamethasone in sesame oil therefore appears to increase UDP-glucuronyltransferase activities catalysing the mono-glucuronidation of bilirubin and the glucuronidation of oestradiol and testosterone in the rhesus-monkey foetus.

Fig. 1. *Analysis of the methyl ester derivatives of the reaction products of the assay for UDP-glucuronyltransferase activity towards bilirubin in foetal-monkey liver pretreated with dexamethasone*

Samples of a liver microsomal preparation from a dexamethasone-treated rhesus-monkey foetus were incubated with bilirubin and digitonin in either the presence (a) or the absence (b) of UDP-glucuronic acid. The reaction products were subjected to alkaline methanolysis and analysed by h.p.l.c. as outlined in the Experimental section. The lines B, M-8, M-12 and D refer to the positions of the peaks of standard solutions of bilirubin, C-8 monomethyl, C-12 monomethyl and dimethyl bilirubin isomers respectively.

Discussion

Our results show that, in liver microsomal fractions from rhesus-monkey foetuses during late gestation, the dexamethasone treatment not only increases UDP-glucuronyltransferase activities towards 2-aminophenol and 1-naphthol, which develop in cluster 1, but also produces large increases relative to control values in activities towards bilirubin, oestradiol and testosterone, which develop in cluster 2. The 25-fold increase in UDP-glucuronyltransferase activity towards bilirubin is particularly significant and does not appear to be due to either artifactual differences in the recovery of activity in the microsomal fraction or false-positive coloration in the enzyme assay. The predominance of bilirubin monoglucuronides over diglucuronides as the main reaction products could, however, be an artifact, because the high substrate concentration relative to enzyme in the assay favours monoglucuronidation (Gordon *et al.*, 1983).

The use of untreated controls prohibits the direct conclusion of whether dexamethasone itself or the vehicle is responsible for stimulating these UDP-glucuronyltransferase activities. However, injection of large volumes of sesame oil (10 µl/g per day intraperitoneally or subcutaneously) into 2–5-day-old rat pups, which are less easily stressed than pregnant monkeys (Poland *et al.*, 1979), had no effect on hepatic UDP-glucuronyltransferase activities towards bilirubin, oestradiol or testosterone, even though the transferase activity towards these substrates are readily inducible by both phenobarbital and dexamethasone in rat pups of this age (J. E. A. Leakey, unpublished work). It is therefore unlikely that the sesame-oil vehicle itself stimulates these UDP-glucuronyltransferase activities in the foetal rhesus monkey.

Our results therefore imply that UDP-glucuronyltransferase activities from both clusters are competent to respond to glucocorticoids prenatally in the rhesus monkey and, in this respect, this species differs from the rat, where analogous dexamethasone treatment fails to increase foetal hepatic UDP-glucuronyltransferase activities belonging to cluster 2.

However, the increased values of these hepatic cluster-2 UDP-glucuronyltransferase activities produced by dexamethasone treatment in the monkey foetuses are still relatively low when compared with corresponding values in liver from adult monkeys, which we have assayed previously (Leakey *et al.*, 1983). Dexamethasone treatment increases UDP-glucuronyltransferase activities towards bilirubin, oestradiol and testosterone to less than 15% of adult values, whereas activities towards 5-hydroxytryptamine and 1-naphthol, which are already present in significant amounts in

liver from control monkey foetuses, are increased to 259% and 120% of adult activities respectively. It is therefore probable that at least some repression of the cluster-2 transferase activities continues during late gestation in the rhesus-monkey foetus, so that these activities appear to be only partially competent to respond to glucocorticoid stimulation *in utero*.

Prenatal development of hepatic UDP-glucuronyltransferase activities towards substrates such as bilirubin and the steroids might be disadvantageous for the mammalian foetus, because glucuronidation is known to decrease the foetal/placental clearance of steroid substrates (Slikker *et al.*, 1982). If such activities were freely responsive to glucocorticoid *in utero*, they would develop in cluster 1 in any species having high endogenous glucocorticoid concentrations in the foetal plasma during late gestation, such as the rat. Our results, however, agree with a previous report (Mitchell *et al.*, 1981) that in the rhesus monkey endogenous glucocorticoid concentrations in the foetal serum are relatively low during late gestation, so that complete resistance to glucocorticoid may not be required to delay development until after birth.

Possibly the two developmental clusters of UDP-glucuronyltransferases exist in this species because endogenous glucocorticoid concentrations during late gestation, although sufficient to evoke development of the cluster-1 activities, are as yet too low to stimulate development of the partially repressed, less sensitive, cluster-2 activities.

Hepatic UDP-glucuronyltransferase activity towards bilirubin is reported to be approx. 5% of adult values in newborn rhesus monkeys and is rate-limiting for the hepatic excretion of bilirubin during the initial stage of physiological jaundice (Gartner *et al.*, 1977). In the same study maternal phenobarbital treatment during the final 6 weeks of pregnancy increased this transferase 3-fold and greatly decreased plasma bilirubin concentrations during the first stage of jaundice. However, phenobarbital failed either to stimulate the transferase or to decrease plasma bilirubin in a 3-week-premature monkey, thus implying that UDP-glucuronyltransferase activity towards bilirubin is not yet competent to respond to phenobarbital stimulation 3 weeks before birth (144 days of gestation). Our findings suggest that glucocorticoids may be more useful than phenobarbital in stimulating the transferase, because activity towards bilirubin is partially responsive to dexamethasone at this stage of gestation. Moreover, if the cluster-2 activities of UDP-glucuronyltransferase in human perinatal liver also prove competent to glucocorticoids during late gestation, then careful glucocorticoid therapy may be of value in managing the jaundiced premature human newborn.

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