Precocious Development *in utero* of Certain UDP-Glucuronyltransferase Activities in Rat Fetuses Exposed to Glucocorticoids

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Received January 19,1977

Summary: The first precocious development of UDPglucuronyltransferase in the mammalian fetus *in utero* by a known compound of endogenous origin is described. Intraperitoneal injection of cortisol (8 mg) into maternal rats on days 14 and 15 of gestation stimulated fetal-liver transferase activity from near zero to ½ maternal levels by day 17; 0.3 mg dexamethasone, possessing a longer biological half-life, raised activity to full maternal level by day 16. In controls, injected with solvent only, fetal-liver transferase remained low on day 16. With both glucocorticoids, transferase stimulation was dose-dependent. Transferase activities were assayed in a range of digitonin concentrations from zero to above optimal for enzyme activation. Activities stimulated were towards *o*-aminophenol, *p*-nitrophenol, 1-naphthol and serotonin. Activities towards bilirubin, morphine and testosterone were not stimulated. The former group of activities are stimulated by glucocorticoids in culture and normally reach approximate adult levels just before birth; the latter group are not so stimulated on culture and normally reach adult levels after birth. Implications of these findings are discussed.

Development of UDP-glucuronyltransferase (E.C. 2.4.1.17), the major phase 2 detoxicating enzyme in mammals, has been much studied (see 1). Although its perinatal rise can be stimulated somewhat by treatment of neonate or pregnant mother with xenobiotics such as phenobarbital (2,3), this increase in fetal liver appears restricted to 2-3 days before term or when the enzyme activity is already demonstrably rising; moreover it is of low degree and does not throw light on the mechanisms initiating the normal perinatal rise *in utero*.

We have recently reported precocious development of this enzyme activity from virtually zero to adult levels in chick-embryo liver exposed in culture or *in ovo* to certain 11β-hydroxysteroids (4), and in explants of 14-day fetal rat liver cultured in a protein-free defined medium

ISSN 0006-291X

containing these glucocorticoids(5). We suggested (5) that glucocorticoids might initiate the surge of UDP-glucuronyltransferase activity occurring *in utero*.

We now report the first evidence to show that these compounds are able to stimulate precocious development of the enzyme in fetal rat liver *in utero*. The enzyme activity stimulated appears to deal with those substrates, endogenous or xenobiotic, glucuronidated by what may be termed the 'late fetal' cluster of transferase activities in the rat, and not with those glucuronidated by the later-developing 'neonatal' cluster.

MATERIALS AND METHODS

Rats were Wistar strain, time-mated. Dexamethasone, cortisol and all substrates were from Sigma. Dexamethasone or cortisol were injected i.p. in 0.1 ml arachis oil at times stated.

UDPglucuronyltransferase activities assayed were towards the following substrates and were as described in the appropriate references: *o*-aminophenol (4), *p*-nitrophenol (4), morphine (6), 1-naphthol (7), bilirubin (8), testosterone (9), serotonin (J.E.A. Leakey, unpublished work). Assays for each substrate in each experiment were conducted over a range of digitonin concentrations from zero to optimal activation of the enzyme, to allow for age-dependent change in activation characteristics (5).

RESULTS AND DISCUSSION

Glucocorticoids cross the rat placenta (10). Injection of glucocorticoids into the mother should therefore expose the fetus to these compounds. The long-acting synthetic glucocorticoid dexamethasone, found effective in inducing the transferase in culture (5), was employed first; being less likely to suffer inactivation by the mother (11), it should provoke a greater response than the natural glucocorticoids.

This proved to be so. When dexamethasone (0.3 mg) was injected into maternal rats on days 14 and 15 of gestation, fetal-liver transferase was

stimulated from near zero to adult levels. For example, if activities are expressed as nmol o-aminophenol glucuronidated per mg protein h^{-1}, they rose from <2.0 to 30.8 of these units by day 17. Adult male rat values were 30-40. Livers from similar fetuses whose mothers received arachis oil only, possessed the usual low (e.g. 2.4 - 2.7) levels of these units on day 17. Cortisol, injected similarly at 8 mg, also stimulated the fetal-liver transferase but, as expected, to a lesser extent (e.g. 7.0 - 7.6 units).

Transferase stimulation was linearly dose-dependent, with both dexamethasone and cortisol. For example, with 0, 100, 200 and 300 μg dexamethasone injected on days 14 and 15, fetal-liver transferase activities on day 17 were respectively 0.6, 9.1, 15.2 and 24.4 of the above units.

The degree of latency of UDP-glucuronyltransferase *in vivo* is not yet known. It is believed (7, 12, *see* 13) to be somewhat higher than in fresh unactivated preparations and to be activated *in vivo* by UDP-*N*-acetylglucosamine. We found transferase development to be stimulated *in utero* by glucocorticoids when measured at several stages of activation by digitonin, from zero to optimal; it was also stimulated to some extent when measured with UDP-*N*-acetylglucosamine (2 mM) in the assay.

We therefore suggest that in mammalian fetal liver, the natural perinatal development of certain UDPglucuronyltransferase activities is brought about by glucocorticoids. This is consistent with the peak in corticosterone levels seen in fetal rat plasma on day 19 and with the increase in maternal plasma on day 18 (14, 15, 16). This development would require amino acid incorporation into protein, following glucocorticoid action at the liver itself, as demonstrated in the experiments with fetal rat-liver explants (5).

However, this mechanism need not operate for UDPglucuronyltransferase activities to all substrates. We have studied developmental rates of the enzyme in our rat colony to several substrates, and divided them into 'late foetal' and 'neonatal' clusters according to the terminology of

Table 1

Precocious development of certain UDPglucuronyltransferase activities in fetal rat liver *in utero* following injection of dexamethasone

Substrate	nmol glucuronide formed mg protein^{-1} hr^{-1}		
	(a)	(b)	(c)
o-aminophenol	0.3, 0.6	18.4, 20.4, 24.4	15.4, 13.0
p-nitrophenol	53.4, 44.5	145, 180, 138	132, 205, 252
1-naphthol	17.0, 12.0, 10.0	78.0, 122.0, 100.0	96.0
serotonin	22.0, 18.0	83.4, 81.6, 78.0	90.0, 87.0
bilirubin	0, 0	0.4, 0	13.0, 18.4
morphine	0, 0	0, 0	170, 230
testosterone	1.0, 0.4	0.2, 0.3	68.0, 55.0

Pregnant rats were injected i.p. on days 14 and 15 of gestation with 300 µg dexamethasone in arachis oil; control pregnant rats were injected with arachis oil only. Transferase activities were assayed on day 16 in (a) fetal liver from control mothers, (b) fetal liver from treated mothers, (c) liver from control mothers. Each result under (a) and (b) represents activity of pooled fetal livers from one mother. Assays as in text; results are from optimally-activated preparations.

Greengard (17), depending on their attaining approximately adult levels of activity before or after birth respectively (18). We now find that dexamethasone injection precociously stimulates activities to substrates in the 'late foetal' cluster (e.g. *o*-aminophenol, *p*-nitrophenol, 1-naphthol, serotonin) but not to those in the 'neonatal' cluster (e.g. bilirubin, morphine, testosterone) (Table 1). These findings *in utero* parallel the effect of dexamethasone on transferase activities in cultured fetal-liver explants, where activities to *o*-aminophenol, *p*-nitrophenol, 1-naphthol and serotonin are precociously stimulated but activities in the 'neonatal' cluster are not (5, 18).

Whatever light these observations may throw on the heterogeneity of the transferase (see 1), they suggest that in the Wistar rat not only bilirubin (5) but other relatively late-developing transferase activities require factors additional to, or different from, glucocorticoids for their development.

We thank the M.R.C. for a grant and Mrs Agnes Donald for skilled help.

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