PRECOCIOUS DEVELOPMENT OF UDP-GLUCURONYLTRANSFERASE ACTIVITY IN CULTURED FETAL RAT LIVER BROUGHT ABOUT BY GLUCOCORTICOIDS AND REQUIRING AMINO ACID INCORPORATION INTO PROTEIN

Graham J. Wishart and G.J. Dutton

Department of Biochemistry, The University,
Dundee DD1 4HN, Scotland.

Received October 18,1976

Summary: Fetal-rat liver explants cultured in a defined protein-free medium containing dexamethasone, corticosterone or cortisol (all 2 μM) exhibit precocious development of UDPglucuronyltransferase activity to o-aminophenol. Transferase activity in 14-day fetal livers cultured with the glucocorticoids for 3 days rises from virtually zero to 5 times the activity seen in fresh 17 day fetal liver. With 15-day fetal livers, precocity was also observed, but to a lesser degree. Precocity always required addition of glucocorticoids, though explants were viable without them. Protein synthesis, not activation, was probably involved, for assays were performed in a range of digitonin concentrations to ensure 'optimal' activation; also, precocious development of transferase activity and uptake of [^{14}C]-leucine into protein exhibited parallel behaviour during inhibition by, and recovery from, cycloheximide-pulsing. This is the first demonstration of a protein-synthesis-dependent stimulation of fetal mammalian UDPglucuronyltransferase by known compounds of endogenous origin. Results with other substrates are discussed.

INTRODUCTION

UDP-Glucuronyltransferase (E.C. 2.4.1.17) is the major 'Phase 2' detoxicating enzyme in mammals. It is present at very low levels in fetal tissues, rising perinatally to adult values at a rate depending on species and substrate. Its delayed appearance gives rise to toxicity from a wide variety of drugs, pollutants and routine metabolites. The mechanism of its perinatal development is therefore of great interest.

We have previously shown that, in chick-embryo liver, the activity of this enzyme towards o-aminophenol can be precociously increased from virtually zero to adult levels by treatment in ovo with corticotropin or certain 11 β-hydroxy steroids (1,2) and have suggested that this mechanism accounts for the natural surge of the enzyme at hatching.

Studies with fetal mammalian liver have been hampered by the marked, and age-dependent, latency of the transferase from that source (3,4,5). Any observed stimulation of transferase could have arisen from accidental activation of the enzyme during assay rather than from previous exposure to stimulating factors. Again, these stimulating factors might activate a latent fetal transferase rather than induce synthesis of new enzyme.

We have recently observed (6) that development of both latent and activated transferase activity in cultured fetal liver requires certain glucocorticoids. We now report the first demonstration of precocious development of UDPglucuronyltransferase activity in fetal mammalian liver due to action of known compounds of endogenous origin, and offer evidence that protein synthesis, possibly of the transferase, is required.

MATERIALS AND METHODS

Organ culture of fetal Wistar rat liver was as described for chick embryo liver by Burchell et al. (7). UDPGlucuronyltransferase activity toward o-aminophenol was assayed in homogenates of fresh and cultured liver as described previously (2). In each experiment the homogenate was incubated in a series of digitonin concentrations over 0-0.2% (w/v), and 'latent' and 'optimally activated' activities of the enzyme were noted; the optimum was always broad at 0.08-0.16%. Pulsing with cycloheximide and incorporation of [^{14}C]-leucine was as described by Burchell et al. (7). Dexamethasone, corticosterone, cortisol and cycloheximide were from Sigma.

RESULTS AND DISCUSSION

As previously found (8), fetal rat liver transferase activity towards o-aminophenol remained low until birth, if homogenates were not treated with digitonin. If homogenates were treated with optimal concentrations of digitonin for activation, activity was negligible until after day 17, when it rose progressively, to reach 5 times the 'optimally-activated' maternal-liver transferase activity on day 21. The following results are with

Table 1. Development of UDPglucuronyltransferase activity in fetal rat liver

Source of liver	nmol o-aminophenyl-glucuronide formed mg. protein^{-1} hr^{-1}
(a)	
14 day fetus	< 2.0
17 day fetus	6.1 ± 1.1 (4)
14 day fetus + 3 days' culture with dexamethasone	31.5, 30.5
14 day fetus + 3 days' culture without dexamethasone	2.8, 2.2
(b)	
15 day fetus	3.9 ± 1.0 (4)
18 day fetus	17.7 ± 4.8 (3)
15 day fetus + 3 days' culture with dexamethasone	28.3 ± 4.1 (12)
15 day fetus + 3 days' culture without dexamethasone	3.0 ± 1.7 (4)

Culture and assay methods as in text. Final concn. of dexamethasone was 2 μM. Enzyme activity shown is at optimal activation with digitonin (see text). Results are from a typical run of experiments, with S.E.M. shown and number of cultures in parentheses. Each culture was from a pool of at least 2 litters.

'optimally-activated' transferase preparations, but the pattern was evident also with preparations untreated with digitonin.

When fetal-liver explants were cultured in a chemically-defined (non-protein) medium containing 2 μM dexamethasone, corticosterone or cortisol, transferase activity increased with time of culture, outstripping the development noted *in utero*. The younger the liver at onset of culture, the more obvious was the precocious development of transferase activity (Table 1). In control cultures without added glucocorticoids, activity remained negligible; these explants were still viable, for on transfer after 2 days to a dexamethasone-containing medium, they exhibited a similar surge in activity to that of fresh tissue.

Table 2. Effect of cycloheximide-pulsing on the development of UDPglucuronyltransferase activity in dexamethasone-treated organ cultures of fetal rat liver

Days in culture	nmol o-aminophenylglucuronide formed mg protein^{-1} hr^{-1}	
0	3.9 ± 1.0 (4)	
1	6.5 ± 0.7 (3)	
	(a)	(b)
2	20.8 ± 2.3 (6)	11.2 ± 0.7 (3)
3	28.3 ± 4.1 (12)	15.9 ± 2.9 (8)
4	34.5 ± 2.6 (6)	26.5 ± 2.7 (5)
5	41.3 ± 5.8 (3)	34.6 ± 1.7 (3)

Culture and assay methods as in text. Livers were from 15-day fetuses. After 24 h of culture, samples (b) were pulsed for 5 hr with 3.5 μM cycloheximide in the culture medium, washed, and returned to fresh medium; samples (a) were treated identically without cycloheximide. S.E.M. is shown; number of samples in parentheses.

Increased activity appeared due to induction, not activation. It was always additive to the activation by digitonin throughout the range of digitonin concentrations used. Moreover, when cultures were pulsed with cycloheximide, transferase development was inhibited, recovering subsequently and within 2 days resuming the rate apparent in controls without cycloheximide (Table 2). Uptake of [^{14}C]-leucine into protein was inhibited 67% during the pulse, and likewise recovered to control rates within 2 days.

Although glucocorticoids precociously increase the proportion of hepatocytes in fetal liver (9), the observed increase in transferase activity is much greater than can be attributable to cell-compositional changes (see 10). Assuming that leucine incorporation involves synthesis of the enzyme itself, glucocorticoids appear to induce precocious development in culture of fetal rat liver UDPglucuronyltransferase activity towards o-aminophenol. Fetal rat liver glycogen synthetase (see 9) and UDPglucuronyltransferase

activity towards serotonin (6), which *in vivo* develop concurrently with this activity, similarly depend upon glucocorticoids in culture. Development of transferase activity to bilirubin, however, which occurs in rat only after birth, has not yet been increased by glucocorticoids, and may require a different or additional stimulus. The glucocorticoids appear to induce selectively certain enzymes appearing *in utero* at day 18. As at that time the endogenous circulating steroids increase markedly (11), the above findings may be relevant to the situation *in vivo*.

Acknowledgements: We thank the M.R.C. for a grant, Mrs Agnes Donald for skilled assistance, and Dr Julian Leakey for stimulating discussion.

References

1. Wishart, G.J. and Dutton, G.J. (1975) Biochem.J. 152, 325-331.
2. Leakey, J.E.A., Wishart, G.J. and Dutton, G.J. (1976) Biochem.J. 158, 419-426.
3. Dutton, G.J. and Burchell, B. (1974) Biochem.Soc.Trans. 2, 1176-1179.
4. Dutton, G.J., Wishart, G.J., Leakey, J.E.A. and Goheer, M.A. in Proc.Sympos. on Drug Metabolism, Guildford, 1976, ed. D.V. Parke and R.L. Smith. *Taylor and Francis, Basingstoke, in press.*
5. Leakey, J.E.A. and Donald, A.M. (1976) Biochem.Soc.Trans. 4, in press.
6. Wishart, G.J., Goheer, M.A., Donald, A.M. and Dutton, G.J. (1976) Biochem.Soc.Trans. 4, 1071-1073.
7. Burchell, B., Dutton, G.J. and Nemeth, A.M. (1972) J.Cell Biol. 55, 448-456.
8. Dutton, G.J. (1964) Proc.European Soc. for Study of Drug Toxicity 4, 121-129.
9. Jacquot, R.L., Plas, C. and Nagel, J. (1973) Enzyme 15, 296-303.
10. Greengard, O. (1971) Essays Biochem. 7, 159-205.
11. Cohen, A. (1973) Horm.Metab.Res. 5, 66.