RELATION BETWEEN THE INDUCTION OF HYDROXYLATION AND OF GLUCURONIDATION IN CHICK LIVER

B. BURCHELL, G. J. WISHART and G. J. DUTTON

Department of Biochemistry, Medical Sciences Institute, The University, Dundee DD1 4HN, Scotland

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1. Introduction

Hydroxylation of drugs or drug-metabolites frequently precedes their glucuronidation [1]. Both processes occur in liver endoplasmic reticulum and both are increased in vivo following administration of barbiturates or polycyclic hydrocarbons [2–4]. Their metabolic relationship is therefore of interest [5–7]; their induction has been thought of as coupled, hydroxylation providing inducers of subsequent glucuronidation [5,6].

Enzymes for both hydroxylation and glucuronidation can be induced in cell- or organ-cultured liver. Induction of hydroxylating enzymes requires phenobarbital [8] in the culture medium. UDP-glucuronyltransferase (EC 2.4.1.17) is induced spontaneously in cultures of chick embryo liver [9,10]; phenobarbital enhances this induction in cultures [11] and increases the enzyme's activity in ovo from zero to very high levels [12].

Hitherto the relationship of hydroxylating and glucuronidating enzymes has not been examined in cultured systems. These systems allow manipulation of the environment, and we report conditions which appear clearly to separate induction of glucuronidation from that of hydroxylation.

2. Methods

Organ and cell cultures of chick embryo liver were prepared as previously described [10,11], phenobarbital (5.5 mM) being present in culture media where required.

Eight-day-old hatched chicks received phenobarbital in drinking water (1 g/l) for up to 7 days before sacrifice. Embryonated White Leghorn eggs were injected via the air space with 10 mg of phenobarbital in 0.3 ml of 0.9% saline; controls received 0.3 ml saline.

Liver homogenates were prepared by homogenising livers in 9 vol 0.15 M KCl with 6 strokes, and centrifuged at 500 *g* for 3 min; the resulting supernatant was used as enzyme source. Microsomes were prepared from liver homogenates in 9 vol 0.25 M sucrose; the supernatant from centrifugation at 9000 *g* for 20 min was re-centrifuged at 100 000 *g* for 30 min with one washing with 0.15 M KCl. The final pellet was resuspended in 0.15 M KCl before use.

Cytochrome *P*-450 was estimated by the method of Omura and Sato [13] using a splitbeam spectrophotometer [14]. UDP-Glucuronyltransferase activity was determined as described [15,16], using *o*-aminophenol as substrate. NADPH-cytochrome-*c* reductase activity was measured [17] at 20°C, cyanide being added to the assay to inhibit any cytochrome-*c* reduction from mitochondrial contamination; this was checked from O_2 consumption using the oxygen electrode. Benzpyrene hydroxylase was estimated by the method of Nebert and Gelboin [18] and aniline hydroxylase as described by Hilton and Sartorelli [19]. Protein was assayed by Oyama and Eagle's [20] modification of the procedure of Lowry et al. [21].

3. Results

3.1. *Hydroxylation and glucuronidation in vivo*

UDP-Glucuronyltransferase, cytochrome *P*-450 and

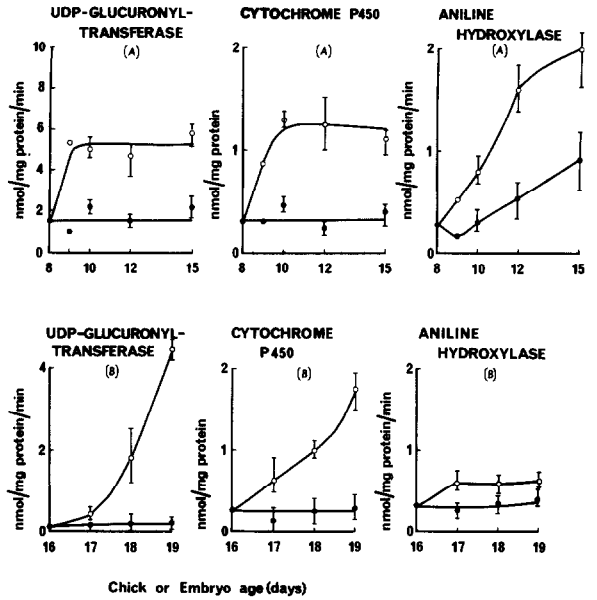

Fig. 1. Drug-metabolising enzyme levels in liver microsomes from control (•) and phenobarbital treated (○) animals: Upper graphs (A), hatched chicks; lower graphs (B), chick embryos. Ranges shown are from at least 3 experiments per point.

aniline hydroxylase activities were measured in microsomes from fresh liver of hatched 8 → 15-day chicks which had been pretreated with phenobarbital. Liver weight and protein content increased 24% and 17% respectively. All these enzyme activities increased approximately 3–4-fold (fig. 1). More rapid induction of any one enzyme was not evident.

3.2. *Hydroxylation and glucuronidation in ovo*

Levels of cytochrome *P*-450, UDP-glucuronyltransferase and aniline hydroxylase were measured in parallel in microsomes from 16 → 19-day chick embryo liver exposed in ovo to phenobarbital (fig. 1). UDP-Glucuronyltransferase increased some 20-fold, aniline hydroxylase 2–3-fold and *P*-450 some 9-fold. Benzpyrene hydroxylase increased only 70% above control levels and NADPH cytochrome-*c* reductase was not increased. Similar results were obtained in homogenate preparations. Therefore under these conditions glucuronidation is induced to a much greater extent than hydroxylation.

3.3. *Hydroxylation and glucuronidation in cultured embryo liver*

On culture of 11-day chick embryo liver, UDP-glucuronyltransferase activity increased steadily whereas aniline hydroxylase activity decreased from low initial values to zero (fig. 2). Cytochrome *P*-450, which exists at adult levels in fresh chick embryo liver, fell to 10% of this initial level after 6 days of culture. High levels of NADPH-cytochrome-*c* reductase also fell to approximately 20% of the fresh value during the culture period studied. Phenobarbital added to the culture medium enhanced UDP-glucuronyltransferase; it did not obviously retard the decline of the hydroxylation system, except that of cytochrome *P*-450.

In fresh 5-day embryo-liver homogenates (fig. 2) no

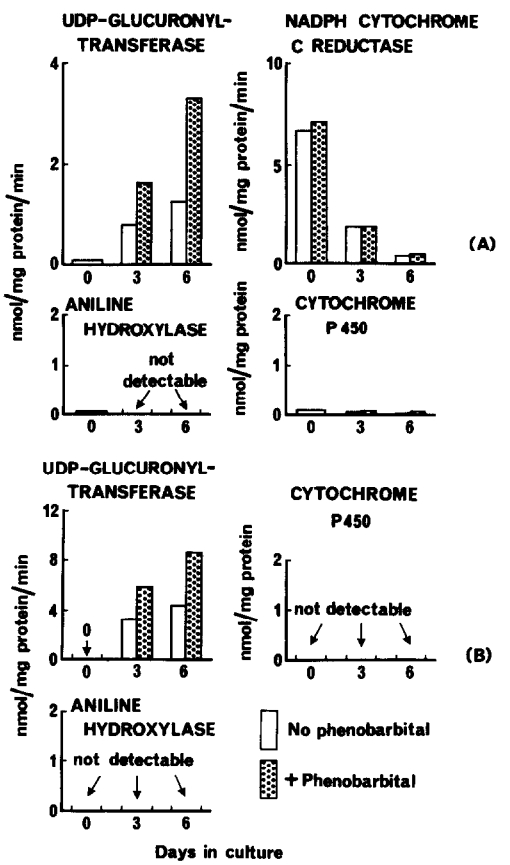

Fig. 2. Drug-metabolising enzyme levels in control and phenobarbital-exposed embryo liver cultures. Upper graphs (A), 11-day embryo livers, lower graphs (B), 5-day embryo livers. Means are shown from at least 3 observations.

UDP-glucuronyltransferase or aniline hydroxylase and no cytochrome *P*-450 were detected. On culture, aniline hydroxylase and cytochrome *P*-450 remained undetectable, but UDP-glucuronyltransferase rose from initial zero levels to values up to 3 times fresh adult levels. With phenobarbital in the culture medium, cytochrome *P*-450 and aniline hydroxylase still remained undetectable, although UDP-glucuronyltransferase was enhanced to 5 times adult levels. Rapid initial induction and breakdown of aniline hydroxylase appeared unlikely, for at 24 hr of culture no activity could be detected.

In cell-cultures from 13-day embryo liver, benzpyrene exhibited some 40% adult levels. After 2 days of culture, under conditions which allowed 10→ 36-fold increase in UDP-glucuronyltransferase activity, benzpyrene hydroxylase declined to 5% of its initial activity; phenobarbital retarded this decline.

4. Discussion

The above results demonstrate that UDP-glucuronyltransferase activity can be increased, even from zero levels, independently of the activity of the hydroxylation enzymes studied. Depending on conditions chosen, hydroxylase activity or cytochrome *P*-450 content may increase to a lesser extent than UDP-glucuronyltransferase, may fall, or may never become detectable. As increase in UDP-glucuronyltransferase activity under these conditions of culture depends upon protein synthesis, then presumably synthesis of glucuronidating and hydroxylating enzyme activities are controlled by different mechanisms; this possibility was suggested by Vainio et al. [22] from in vivo inhibition of the two systems, and by Hietanen and Vainio [23] from interspecies variation. It is consistent with the differential action in vivo of chlorpromazine, inducer of mixed-function oxidases [24] but not of UDP-glucuronyltransferase [25], and with that of cortisone in ovo, which induces aniline hydroxylase but not the transferase (G. J. Wishart, unpublished work). Thus there seems no reason to believe that induction of hydroxylating enzymes necessarily precedes that of UDP-glucuronyltransferase [5,6] by providing substrates essential to induce the latter enzyme; nor, even if glucuronidation and hydroxylation are intimately linked in the endoplasmic reticulum membrane [7], need induction of one necessarily involve induction of the other.

Only a limited number of substrates were investigated. However, transferase activities towards all xenobiotic substrates we have examined are inducible by conditions which do not induce aniline or benzpyrene hydroxylases. Moreover, when such marked divergence can be demonstrated even on one substrate from each system, then inductions of the two systems are probably not linked.

Hydroxylating-enzyme activity increased in foetal rat liver-cell cultures in response to phenobarbital [8], but lack of such response in the embryonic avian-liver cultures employed here is not due to general cellular degeneration. These cells in cell culture mature and divide [10], and in the 5-day chick-embryo liver organ cultures (examined under the electron microscope) develop into mature hepatocytes [26], maintaining their initial levels of glycogen synthetase, glycogen phosphorylase [27], serine hydroxydemethylase and ribonuclease [11]. Tyrosine aminotransferase also is maintained in these 11-day embryo-liver cultures (B. Burchell, unpublished work).

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