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Precocious Development of Cytochrome *P*-450 in Neonatal Rat Liver after Glucocorticoid Treatment

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Intraperitoneal injection of neonatal rats with glucocorticoid hormones causes precocious development of hepatic cytochrome *P*-450. Glucagon injection fails to stimulate this cytochrome *P*-450 development. Adult liver cytochrome *P*-450 is less responsive to glucocorticoid stimulation than is that of neonatal rat liver. Adrenalectomy of prematurely delivered neonatal animals prevents the early postnatal development of cytochrome *P*-450. Glucocorticoids failed to increase cytochrome *P*-450 concentrations in foetal rat liver. These findings imply that, although glucocorticoids are mandatory regulatory factors controlling cytochrome *P*-450 development, they are not themselves the 'trigger' initiating onset of that development.

Foetal and neonatal mammals possess limited capacity to metabolize drugs and environmental compounds, mainly owing to low activities or absence of enzymes such as mixed-function oxidases (Fouts & Adamson, 1959; Jondorf *et al.*, 1959) functioning via cytochrome *P*-450, which in the rat develops to adult concentrations in the early postnatal period (Basu *et al.*, 1971; Lucier *et al.*, 1975). The physiological factors initiating and controlling the development of this cytochrome remain obscure (see Short *et al.*, 1976), though maternal progestins (Feuer & Liscio, 1969), somatotropin (Wilson, 1970) and glucocorticoids (Mukhtar *et al.*, 1974) have all been suggested to influence the overall development of mixed-function oxidase activity.

In certain mutant mice, cytochrome *P*-450 and several other 'early-neonatal' enzymes, including tyrosine aminotransferase and L-serine de-ethylase, fail to appear at birth (Thaler *et al.*, 1976), which suggests a deficiency of a common mechanism controlling the development of these enzymes.

As hepatic tyrosine aminotransferase activity in the neonatal rat is stimulated by glucagon and glucocorticoid hormones (Greengard, 1969; Holt & Oliver, 1969), we have investigated effects of these hormones on cytochrome *P*-450 development at various ages.

Materials and Methods

Rats of the CD strain (Charles River Laboratories) were injected intraperitoneally with various hormones

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(Sigma Chemical Co., St. Louis, MO, U.S.A.) suspended in 0.9% NaCl, at ages and doses shown in the text. Controls received 0.9% NaCl alone. Foetal rats were treated with dexamethasone by intraperitoneal injection of the mother.

For the adrenalectomy experiments, foetal rats were delivered by uterine section on the 22nd day of gestation, 5–10h before the normal birth time; adrenalectomy was then performed by the method of Holt & Oliver (1971). In 'sham-operated' controls the adrenals were exposed but not removed. Surgery was complete within 40min of delivery. The animals were kept warm for 10h, then fostered until assay 4 days later.

Livers were homogenized in 150mM-KCl containing 1.25mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] buffer, pH7.6, and centrifuged at 11000g for 20min. The resulting supernatant was centrifuged at 150000g for 30min and the supernatant obtained was assayed for tyrosine aminotransferase activity. The microsomal pellet was washed and resuspended in 50mM-potassium phosphate buffer, pH7.6, for cytochrome *P*-450 determination.

Tyrosine aminotransferase activity was assayed as described by Diamondstone (1966), but with an incubation mixture containing 2.7mM-L-tyrosine, 1.2mM-pyridoxal phosphate, 1.5mM-diethyldithiocarbamic acid and 2mM-2-oxoglutarate. Cytochrome *P*-450 (not differentiated here into *P*-448 and *P*-450 species) was assayed by the method of Omura & Sato (1964) with an Aminco DW-2 double-beam spectrophotometer. Protein was assayed by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Results

Cytochrome *P*-450 was detected in late-foetal rat liver (19–21 days) at between 0.06 and 0.13 nmol/mg of microsomal protein. Its concentration (measured daily) increased linearly from birth, reaching values of 0.7–0.8 nmol/mg of microsomal protein by the 10th postnatal day. It then remained within these values until after weaning. Tyrosine aminotransferase activity developed much more rapidly, and increased from late-foetal values of 2–4 nmol/min per mg of protein to 40–50 nmol/min per mg of protein at 12 h after birth, before decreasing to adult values of 20–25 nmol/min per mg of protein by the 2nd postnatal day.

Administration of the glucocorticoids dexamethasone acetate or corticosterone acetate to 2½-day-old neonatal animals precociously stimulated the rate of development of cytochrome *P*-450 (Table 1). A similar stimulation was observed on the basis of liver wet weight. As expected, this treatment stimulated tyrosine aminotransferase activity. Injection of glucagon also increased tyrosine aminotransferase activity, but slightly decreased the cytochrome *P*-450 concentration. Injection of NaCl caused no significant change in either cytochrome *P*-450 concentration or tyrosine aminotransferase activity from values in untreated neonatal animals.

Adrenalectomy of 5–10 h-premature neonatal animals inhibited development of their hepatic cytochrome *P*-450. Its hepatic concentration in adrenalectomized 4-day-old neonatal animals (Table 1) was not significantly higher than in 21-day foetuses (Table 2). The surgical procedures also, to a smaller extent, inhibited cytochrome *P*-450 development, as the 'sham-operated' values were lower than those of untreated or NaCl-treated 4-day-old neonatal animals (Table 1).

Response of cytochrome *P*-450 to glucocorticoids was examined from foetus to adult (Table 2). Dexamethasone pretreatment decreased the hepatic cytochrome *P*-450 concentration in the foetus, but increased it in the newborn rat. Dexamethasone pretreatment also stimulated cytochrome *P*-450 concentrations in 12½–14-day-old rat pups, where control values approached those of adult females. Liver from adult rats was less responsive, as the cytochrome *P*-450 concentration was increased only a small amount (as first shown by Tredger *et al.*, 1976) when large doses of dexamethasone (Table 2) were injected every 12 h. Single doses (35–100 mg/kg) were ineffective in increasing the cytochrome *P*-450 concentration, though they more than doubled tyrosine aminotransferase activity.

Discussion

Even though both cytochrome *P*-450 and tyrosine aminotransferase begin to develop at birth, the natural development of the cytochrome is slower than that of the transferase, and unlike it cytochrome *P*-450 does not respond to glucagon. But cytochrome *P*-450 does precociously develop in response to glucocorticoids, which implies involvement of endogenous glucocorticoids in its natural development. However, cytochrome *P*-450 can be induced by many hydrophobic compounds (see Conney, 1967). The high glucocorticoid doses required to stimulate the cytochrome could thus imply that the glucocorticoids act by a 'xenobiotic-type' of induction process unrelated to the cytochrome's natural development. Against this, the relative insensitivity of the adult liver cytochrome to dexamethasone stimulation, compared with that of the neonatal animal, does not suggest a 'xenobiotic-type' of induction. Polycyclic hydrocarbons, for instance, are known to induce

Table 1. *Effects of hormone treatment and adrenalectomy on neonatal rat liver cytochrome P-450 concentration and tyrosine aminotransferase activity*

Neonatal rats were treated with the hormones at the doses shown (per kg body wt.) at 2½, 3 and 3½ days *post partum*. Dexamethasone-treated animals received only a single injection at 2½ days. Adrenalectomy was performed on premature neonatal animals as described in the Materials and Methods section. Livers were assayed at 4 days *post partum*. Results are expressed as means ± S.E.M. with the numbers of animals used in parentheses. For values significantly different from controls: **P* < 0.001; ***P* < 0.01; ****P* < 0.05.

Treatment	Dosage (mg/kg per 24 h)	Cytochrome <i>P</i> -450 content (nmol/mg of protein)	Tyrosine aminotransferase activity (nmol/min per mg of protein)
NaCl	5	0.294 ± 0.010 (11)	19.5 ± 2.8 (11)
Glucagon	50	0.242 ± 0.010 (6)**	68.3 ± 3.5 (6)*
Corticosterone	100	0.488 ± 0.024 (5)*	72.8 ± 13.3 (5)*
acetate	50	0.348 ± 0.021 (4)***	64.5 ± 15.8 (4)*
	25	0.299 ± 0.020 (4)	60.2 ± 12.1 (4)*
Dexamethasone acetate	35	0.773 ± 0.097 (5)*	82.2 ± 14.2 (5)*
'Sham-operated' controls		0.214 ± 0.029 (8)	26.2 ± 3.7 (8)
Adrenalectomized		0.114 ± 0.008 (7)**	50.3 ± 11.6 (7)***

Table 2. Effect of age on dexamethasone-induced stimulation of rat liver cytochrome P-450 concentration and tyrosine aminotransferase activity

The rats were pretreated with either NaCl or dexamethasone acetate at the doses shown, 1½ days before assay. Foetal rats were treated by intraperitoneal injection into the mother. Results are expressed as means ± S.E.M. with the numbers of animals used in parentheses.

Age at assay (days)	Dexamethasone treatment (mg/kg body wt.)	Cytochrome P-450 content (nmol/mg of protein)	Percentage of control values and significance
21 (foetal)	NaCl	0.111 ± 0.018 (8)	—
	100	0.060 ± 0.008 (8)	64% $P < 0.05$
1½	NaCl	0.216 ± 0.019 (11)	—
	35	0.617 ± 0.090 (12)	281% $P < 0.001$
14	NaCl	0.702 ± 0.031 (7)	—
	35	1.143 ± 0.026 (4)	163% $P < 0.001$
	100	1.778 ± 0.069 (6)	253% $P < 0.001$
Adult female (> 60)	NaCl	0.782 ± 0.019 (8)	—
	100	0.778 ± 0.050 (6)	99% Not significant
	300*	1.219 ± 0.046 (6)	156% $P < 0.001$
Adult male (> 60)	NaCl	1.186 ± 0.030 (10)	—
	100	1.270 ± 0.104 (5)	107% Not significant
	300*	1.593 ± 0.089 (5)	134% $P < 0.001$

* Three injections of 100 mg of dexamethasone acetate/kg given 1½, 1 and ½ days before assay.

mixed-function oxidase activity to a greater extent in liver from adult rats than in that from neonatal animals (Nebert & Gelboin, 1969).

Moreover, the adrenalectomy experiments suggest that adrenal hormones are necessary for the initial postnatal development of cytochrome P-450; livers from adrenalectomized neonatal animals possess cytochrome P-450 concentrations no higher than those of late-foetal rat liver. These findings strongly suggest that the natural postnatal development of cytochrome P-450 is indeed dependent on glucocorticoids.

However, dexamethasone fails to cause premature development of cytochrome P-450 in foetal rat liver. This is not due to inability of the steroid to reach the foetal liver, for such treatment certainly stimulates other foetal hepatic enzymes (Wishart & Dutton, 1977). Also, endogenous glucocorticoid concentrations are higher in late-foetal plasma than in neonatal plasma (Greengard, 1975; Martin *et al.*, 1977). Clearly, therefore, endogenous glucocorticoids cannot be the 'trigger' initiating the development of cytochrome P-450, as was first suggested (Mukhtar *et al.*, 1974). It is more reasonable to propose that competence of the perinatal cytochrome P-450 to respond to glucocorticoid stimulation is evoked at birth, and that subsequently the cytochrome develops in response to the endogenous glucocorticoid concentrations in the neonatal animal.

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