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PROGRAMMING OF HEPATIC HISTIDASE FOLLOWING PRENATAL ADMINISTRATION OF DIETHYLSTILBESTROL

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SUMMARY

Diethylstilbestrol was demonstrated to be a positive modulator of rat liver histidase in a manner similar to oestradiol-17β. Oral administration of diethylstilbestrol to pregnant rats at the 15th day of gestation and subsequent measurement of the sex-dependent activities of histidase in these offspring revealed no effect on histidase activities in immature male and female and adult male offspring. However, histidase activities of the adult female offspring were decreased and activities approached those of normal adult males. This masculinization of histidase activity can be reversed by exogenously administered estrogen.

INTRODUCTION

One form of hormonal regulation of liver enzyme development occurs through the stimulation of gene expression whereby changes in enzyme activities are evident shortly after hormone treatment. Another form of regulation is initiated during a critical period of sexual differentiation in late prenatal or early postnatal development [1]. Expression of the latter type of hormonal regulation does not occur until after sexual maturation and has been defined as "imprinting" or "programming" of hepatic metabolism [2]. Previous studies have demonstrated programming of rat liver steroid metabolizing enzymes following early postnatal exposure to steroid [2, 3].

Our laboratory is interested in the effect of perinatal exposures of hormonally active xenobiotics on the ontogeny of metabolic competence. We have chosen hepatic histidase as a model system to study environmentally-related alterations in the normal programming process for a number of reasons. Rat liver histidase displays a polyphasic sex-dependent postnatal development [4, 5]. The catalytic activity of this enzyme initially appears shortly following parturition, rises for several weeks, plateaus during the post-weaning period, then increases again during puberty. The post-pubertal increase is greater in the female than the male; adult enzyme levels are attained which are approximately two-fold higher in the female than in the male. As reported in a recent review [5], regulation of the developmental pattern appears to be a function of concerted action of several hormones and nutritional factors acting at specific developmental stages: glucagon (via cAMP), glucocorticoids, and estrogen serve as histidase inducers, and the sex-dependent developmental elevations in histidase catalytic activity are accompanied by increased amounts of enzyme antigen and parallel increases in the rates of enzyme synthesis [6, 7].

Because rat liver histidase has such a complex developmental course that is reversibly modulated by estrogen [8], we have investigated the effect of diethylstilbestrol (DES), a potent synthetic estrogen, on the activity of hepatic histidase. Moreover, we have studied the effect of prenatal exposure to DES on the postnatal development of this enzyme in an attempt to determine if gestational exposure to DES can program hepatic histidase activities.

EXPERIMENTAL

Studies were carried out using birth-dated CD-stock random bred albino rats (Charles River Breeding Laboratories, Inc., Wilmington, MA). The animals were maintained on synthetic diets (Wayne Sterilizable Lab-Blox, Allied Mills, Inc., Selma, NC) and allowed free access to water. Animals were weaned at 21–23 days of age and housed 4 per cage.

DES was administered orally to pregnant females at the 15th day of gestation using cottonseed oil as vehicle via stomach tube. Postnatal injections were given subcutaneously using propylene glycol as vehicle. Exogenously administered compounds were purchased from Sigma Chemical Co., St. Louis, MO.

Animals were decapitated, allowed to bleed and their livers rapidly removed. Twenty-five % liver homogenates in 10 mM Tris, pH 7.2, containing 14 mM $MgCl_2$ and 0.6 M KCl was prepared in a motor driven glass Potter–Elvehjem homogenizer equipped with a Teflon pestle, and spun at 105,000 *g* for 60 min. This high speed supernatant was used for enzyme assays. Bilateral ovariectomies were preformed on 42 day old females using methoxyflurane as inhalation anesthetic.

Histidase was assayed on a recording Gilford spectrophotometer, Model 250, thermostated at 37°C [7]. One unit of enzyme is defined as the amount producing one μmol of urocanic acid/min.

Table 1. Effect of oestradiol-17β and diethylstilbestrol on histidase activity in young male rats and in adult ovariectomized female rats

Expt.	Treatment	Histidase activity (Units/G liver)†	Histidase activity (% controls)
1	Controls (5)	0.19 ± 0.01	100
	Oestradiol-17β (5)	0.34 ± 0.03*	177
	Diethylstilbestrol (5)	0.34 ± 0.03*	175
2	Ovariectomized controls (5)	0.69 ± 0.03	100
	Ovariectomized oestradiol-17β (6)	1.03 ± 0.07*	149
	Ovariectomized Diethylstilbestrol (6)	0.98 ± 0.08*	141

Male rats in experiment 1 received daily subcutaneous injections of propylene glycol (0.1 ml), E_2 or DES (100 μg/kg b.w.) from days 28–35 postpartum. Female rats in experiment 2 were ovariectomized at Day 42 and received propylene glycol (0.25 ml) E_2 or DES as above from day 56–63 pospartum. Numbers in parenthesis represent number of rats used. †Mean ± S.E.M. *$P < 0.01$.

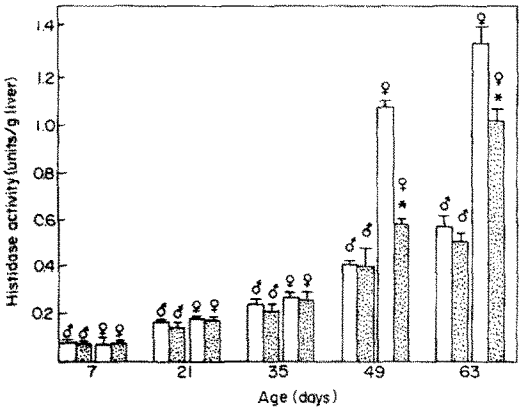

Fig. 1. Postnatal development of hepatic histidase in rats prenatally exposed to Diethylstilbestrol. DES (100 μg/kg b.w.) was administered orally to pregnant females at day 15 of gestation and hepatic histidase was measured in the offsprings. Open bars = control animals; stipled = prenatally exposed to DES. Results are expressed as Mean ± S.E.M. *$P < 0.01$.

RESULTS

Table 1 illustrates the effect of oestradiol-17β (E_2) and DES on rat liver histidase. In experiment 1, using young male rats, E_2 and DES treatment elevate hepatic histidase activity to 177 and 175% respectively of control values. Histidase activities are also elevated following the same DES and E_2 dosage regimen to adult ovariectomized female rats (Table 1).

To investigate the effect of prenatal exposure of DES on postnatal development, we administered a single dose of 100 μg DES/kg b.w. orally to pregnant rats on the 15th day of gestation. Weight gains of treated animals were not significantly different from those of controls at any developmental stage tested, and total and postnatal survival indexes were unaffected using the specified dosage regimen. Histidase activities were measured in the offspring of these treated females. The results in Fig. 1 show no apparent change in histidase activity of control compared to prenatally exposed immature male and female rats (7, 21, 35 days of age) and adult male offspring (through 63 days of age). However, histidase activities of 49 and 63 day old females exposed to DES prenatally were lowered by 46 and 30% from controls.

Exogenously administered E_2 (daily subcutaneous injections from days 42–49 postpartum) to prenatally exposed female offspring demonstrated that these lowered histidase levels could be increased (Table 2)

Table 2. The action of oestradiol-17β on hepatic histidase in prenatally programmed female rats

Expt.	Treatment	Histidase activity (Units/G liver)†	Histidase activity (% controls)
1	Programmed controls (5)	0.58 ± 0.04	100
	+ oestradiol-17β (5)1	0.95 ± 0.03*	163
2	Programmed controls (ovariectomized) (5)	0.64 ± 0.02	100
	+ Oestradiol-17β (5)	1.06 ± 0.06*	160

Propylene glycol or E_2 (100 μg/kg b.w.) was administered subcutaneously to female rats that were prenatally exposed to DES (100 μg/kg b.w.). In experiment 1, treatment was from day 42–49 postpartum. In experiment 2 animals were ovariectomized at day 42 and treated from day 56 to 63 postpartum. Numbers in parenthesis represent number of rats used. †Mean ± S.E.M. *$P < 0.01$.

to activity levels similar to those of normal females (see Fig. 1). Similar results were obtained upon exogenously administering DES; 0.58 ± 0.04 vs 0.99 ± 0.06. Histidase levels of prenatally exposed females that were ovariectomized and given E_2 on the same schedule could also be elevated (160% that of control values, Table 2).

DISCUSSION

Diethylstilbestrol is a nonsteroidal estrogenic substance that has been used as a postcoital contraceptive, an anti-abortion drug, an estrogen replacement, and a growth stimulant in food-producing animals. Since DES is a potent synthetic estrogen, we thought it might be a positive modulator of rat liver histidase. Our data demonstrated that E_2 and DES increased histidase activity to approximately the same extent in both 28 day old males and in adult ovariectomized females.

Increased evidence of clear cell vaginal adenocarcinoma was reported in female offspring of women exposed to DES during pregnancy [9]. This study has stimulated much interest in the effect of DES on prenatally exposed offspring in animal models emphasizing histological and morphological experimentations [10, 11]. However, little attention has been accorded the possibility that DES may have more subtle effects on the postnatal development of specific biochemical systems under well-defined hormonal regulation.

Using rat liver histidase as our model system, we investigated the effect of prenatal administration of DES on postnatal enzyme development. While no change in histidase activity is observed in the male and female young offspring and in adult male rats of DES treated mothers, a significant decrease of histidase activity in the adult female offspring was observed. Since the altered expression of histidase activity was not evident until later in postnatal development it appears that DES administered prenatally has elicited a programmed effect. A mechanism for the programming of sexual differentiation of the brain and hepatic metabolism has been proposed involving alpha-fetoprotein which is produced in large quantities by the fetal and newborn liver [12]. Alpha-fetoprotein is an effective binder of oestradiol in the cerebrospinal fluid and plasma but it binds poorly to testosterone. Testosterone produced by the developing testes reaches the target cells in the brain where it is converted to oestradiol [12, 13, 14]. Oestradiol binds to E_2-receptor in the brain which initiates the process resulting in a male type of sexual differentiation and hepatic metabolism. Female rats and newborn male rats deprived of testosterone (castrated) maintain a female type of metabolism [12]. Feigelson [15, 16] has demonstrated that the pituitary is necessary for complete expression of hepatic histidase activity. While hypohysectomy of young females results in higher histidase activity in the rat liver, induction of histidase is not possible with E_2 in these same hypophysectomized animals. Gustafsson and coworkers [2, 17] have carried out similar studies with steroid-metabolizing enzymes and suggest that these differences are regulated by the hypothalamic-hypophysical axis.

Unlike E_2, DES binds poorly to alpha-fetoprotein [12] and should reach target cells in the developing female brain thereby programming male histidase activities in the resultant adult female. Previous studies have demonstrated postnatal programming of hepatic steroid metabolism following castration and/or androgen treatment of newborn rats [2, 17]. Our studies are unique in two respects; (1) we were able to program hepatic metabolism during the fetal period (it is unlikely that DES would be present in sufficient quantities postnatally since it has a short biological half-life and is rapidly metabolized and inactivated) and [2] we used a non-steroid metabolizing enzyme as our marker. We conclude that DES does indeed exert its programming action on hepatic histidase during the fetal period.

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