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Biochemical Pharmacology, Vol. 28, pp. 1429–1430.
Pergamon Press Ltd. 1979. Printed in Great Britain.

Long term induction of microsomal drug oxidizing system in mice following prenatal exposure to barbiturate

(*Received* 2 *October* 1978; *accepted* 16 *November* 1978)

Rodent neonates have a poor ability to metabolize many drugs including barbiturates [1, 2]. Hart *et al.* [3] have demonstrated that following phenobarbital administration to pregnant female rabbits, the fetuses and the neonates showed microsomal drug oxidizing system activities similar to those found in adults. The increased enzyme activity was accompanied by an increased ability to tolerate and metabolize barbiturates [4]. These studies included only the short term effects and the possibility that the metabolic changes were long lasting was not investigated. This is perhaps since biochemical events resulting from administration of sedative hypnotics to adults are mostly transient. This fact is particularly true for the induction of the microsomal drug oxidizing system [5]. Recently, however, it has been shown that the outcome of prenatal administration of sedative hypnotics lasts into adulthood. Thus, early administration of ethanol and barbiturate induced long lasting neural [6, 7] and behavioral changes in mice [8–10]. Most pertinent, mice which received ethanol prenatally showed a higher activity of hepatic alcohol dehydrogenase (ADH) and microsomal ethanol oxidizing system (MEOS) at adulthood [11]. Consequently, the present experiment was conducted to study the long lasting effects of prenatal administration of a barbiturate on the activity of the microsomal drug oxidizing system in adulthood.

HS mice were used. This strain was derived through crosses of eight inbred strains and was deliberately maintained genetically heterogeneous for many generations [12]. Adult animals which were used as parents in the present experiment were housed in mating groups of two males and five females and maintained under standard laboratory conditions. Females were checked daily at 0800 and those that conceived, as evidenced by the existence of a vaginal plug, were separated from the males and housed with other pregnant females. Phenobarbital (PhB, acid form) administration commenced on gestation day 6 (GD6; the day in which the plug was found was considered GD1) and lasted until parturition (P). The females were then housed in individual cages. From GD6 to P, treated females received milled food (Ralston Purina, St. Louis, MO) containing PhB as their only food source and water, both available *ad libitum*; control females received milled food and water. Dose schedule was as follows: GD 6–9, 0.526 g PhB/kg food; GD 9–18, 3 g/kg; GD 18–P, 1 g/kg. Blood PhB levels were monitored on a sample group on GD 8, 10, 12, 15, 18, and 20. Thus, levels from all periods and doses were studied. The tail blood (100 μl) was taken at 0900, suspended in 50 μl 1M H_3PO_4, the PhB extracted into 5 ml toluene and assayed by gas chromatography on OV 17, after being derivatized on column with 20 μl of tetramethylammonium hydroxide. This method is described elsewhere [13].

In order to control for the possible carry-over effect of PhB on maternal milk production or behavior, half of the offspring born to mothers who received PhB during pregnancy (B) were fostered by lactating control females (C) within 12 hr post partum. Half of the C litters were also fostered by C mothers other than their own. Since preliminary analysis revealed no such carry-over effects on any of the variables studied, the results of the fostered and the non-fostered offspring were pooled. The male offspring which were the subject of the present experiment were weaned on day 23. They were tested for enzyme activity on days 45 to 50. The animals were weighed and decapitated and the livers quickly removed. The activity of the microsomal drug oxidizing system was determined in a supernatant fraction which contained the microsomes and soluble proteins according to the method described by Zannoni [14]. Briefly, livers were washed in 0.25 M ice cold sucrose solution, blotted and weighed. A 20% (w/v) homogenate was prepared with the sucrose in a glass Potter–Elvehjem homogenizing tube and centrifuged at 15,000 *g* for 30 min at 5°. The supernatant (0.3 ml) was added to a 2.2 ml mixture of 6.0 μmoles nicotinamide, 10 μmoles glucose-6-PO_4, 3.0 μmoles *p*-nitroanisol and $MgCl_2$ all made up in 0.07 M monophosphate buffer pH 7.8 and incubated at 37°. The reaction was initiated with the addition of 3 μmoles NADP at 37°. The activity of the enzymes, as indicated by the formation of *p*-nitrophenol, which is the demethylated product of *p*-nitroanisol, was followed spectrophotometrically for 8 min at 420 nm. The protein content of the supernatant was assayed according to the method of Lowry *et*

Table 1. The activity of microsomal drug metabolizing system and seminal vesicle weight in control offspring and offspring whose mother consumed barbiturate during pregnancy

Prenatal treatment	Enzyme activity (ΔOD.10^3/min/mg protein)	Seminal vesicle weight (mg)
Control	2.92 ± 0.24 (11)	98.9 ± 4.6 (14)
Barbiturate	3.84 ± 0.19* (10)	81.6 ± 3.6* (15)

Figures in parentheses represent sample sizes.
* $P < 0.01$, *t* test [16].

al. [15]. Seminal vesicle weight was also taken in a sample group. Sample sizes are given in Table 1.

During the initial feeding period of 0.526 g/kg food blood PhB levels ranged between 0–50 μg PhB/ml blood; 3 g PhB/kg food resulted in 40–200 μg PhB/ml blood. Administration of 1 g PhB/kg food on GD 18–P, when the animals were presumed tolerant, resulted in only traces of PhB in the blood ($n = 19$). Fetal PhB levels were similar to their mothers. Offspring who were exposed to PhB *in utero* (B) had a similar body and liver weight to controls on days 45–50. Protein content per mg liver was also similar in both groups.

The activity of the microsomal drug oxidizing system was measured as ΔOD/min/mg protein. As can be seen in Table 1, B animals had 32 per cent higher activity of microsomal drug oxidizing system per mg protein than controls ($P < 0.01$). Seminal vesicles of B offspring were 17.5 per cent lighter than C ($P < 0.01$).

Barbiturates are mostly oxidized in the hepatic microsomal fraction by the microsomal drug oxidizing system; this system is readily induceable. However, like most inductions of enzyme systems, the increased activity of the microsomal drug oxidizing system is only transient [5]. The significance of the present finding is in demonstrating that when the barbiturate is given early enough, when the liver is still developing the resulting microsomal enzyme induction is *long term* and lasts well into adulthood. This phenomenon seems to be more general and not limited only to barbiturates since in our previous study, prenatal administration of ethanol resulted in long term induction of hepatic microsomal–ethanol oxidizing system (MEOS) and the cytoplasmic alcohol dehydrogenase (ADH), while the activity of acetaldehyde dehydrogenase (AcDH) remained unchanged [11].

Early input of barbiturates also results in a decrease in the size of the seminal vesicles. This change may be linked in causal relationship to the increase in the microsomal enzymes activity. It is well established that microsomal enzymes metabolize steroid hormones including testosterone, and that the induction of the enzyme enhances the testosterone metabolism [17]. Since testosterone level is related to the size of the seminal vesicle [18] it is possible that the higher activity of microsomal drug oxidizing system caused the decrease in the size of the seminal vesicles.

The possibility that prenatal effects of drugs are mediated by malnutrition is particularly important in ethanol research as was previously discussed [19]. Barbiturates, however, have no significant caloric value, and in the present study the weight of the B offspring did not fall behind controls. Indeed, there are indications that barbiturates may act to enhance food intake [20].

Inbred strains of mice provide uniform replicable material for biochemical and pharmacological research. On the other hand each strain represents only many replications of a single genotype. Thus effects of drugs may not be elucidated because of a possible genetic peculiarity of the strain under study (genotype environmental interaction [21]). The heterogenous stock (HS) was derived by crosses among eight inbred strains so that each individual represents genetic uniqueness [12]. Thus, the action of the drug may be observed on a whole spectrum of genotypes.

Prenatal administration of barbiturate also resulted in a variety of long-lasting changes in the CNS and behavior. Among them are the enhanced functional (CNS) tolerance to barbiturate and specific neuronal deficits. These effects, which are the subject of our current investigation, are being reported elsewhere [22, 23].

Acknowledgement—This investigation was supported by USPHS grant DA 1951 and by the Hebrew University–Hadassah grant.

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