useful in delineating the early events in the reaction scheme, because it can be examined in a stable oxyferrous form.

In summary, cyclohexane oxidation is a prototypic monooxygenase activity previously thought to be exclusive to cytochrome P-450 systems. In this report, hemoglobin (Hb) was shown to catalyze the hydroxylation of cyclohexane in a system containing O_2, NADPH, and cytochrome P-450 reductase. The reaction requires each component of the complete catalytic system; omission of Hb or cytochrome P-450 reductase or NADPH resulted in no measurable activity. The reaction is dependent on the O_2 content of O_2/N_2 gas mixtures, and substitution of O_2/CO (20/80) for O_2/N_2 (20/80) caused marked inhibition. The apparent K_m for Hb-catalyzed cyclohexane oxidation is similar to that for the corresponding cytochrome P-450_{LM2}-mediated reaction, but the V_{max} of the Hb reaction is approximately 500 times lower than that for P-450_{LM2}. The basis for this quantitative difference remains to be elucidated.

Acknowledgements—We are grateful to Mr. Mark W. Nowak for technical assistance, to Mr. Wesley Gray for the gas chromatographic/mass spectral analysis, and to Drs. William Fuchsman, Dennis Koop, and Vernon Shoup for critical evaluation of the manuscript prior to submission.

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Biochemical Pharmacology, Vol. 38, No. 1, pp. 204–206, 1989
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

Effects of maternally administered cimetidine during lactation on the development of drug metabolizing enzymes in mouse pups

(*Received* 25 *February* 1988; *accepted* 1 *July* 1988)

The H_2 antihistamine, cimetidine, is a drug of choice in duodenal ulcer and related disorders [1]. Despite the great attention and severe scrutiny to which it has been subjected, its use in the perinatal period is poorly documented except perhaps as it relates to prophylaxis against aspiration of acid stomach contents during obstetric anaesthesia [2]. However, cimetidine is known to cross the placenta and to be excreted in breast milk [3, 4]. It is not known what effects if any, such indirect exposure holds for the breastfed young of treated mothers.

Several cimetidine–drug interactions have been described in animals and man; the vast majority of which occur as a result of inhibition of microsomal oxidation mechanisms [5, 6]. Prolongation of barbiturate sleeping time has been attributed to this ability of cimetidine to inhibit drug metabolism [7]. Cimetidine has also been shown to inhibit aminopyrine *N*-demethylase in rodents [8, 9].

The objective of this study was to investigate the effects if any, of maternally administered cimetidine during lac-

tation on the development of drug metabolizing enzymes in mouse pups using both aminopyrine *N*-demethylase activity and pentobaritone hypnosis as indices.

Materials and methods

The following were obtained from Sigma Chemical Co.: cimetidine, nicotinamide adenine dinucleotide phosphate (NADP), bovine serum albumin (BSA) and glucose-6-phosphate dehydrogenase. Pentobarbital sodium was from May and Baker while all other chemicals were of analaR grade and obtained from BDH Chemicals Ltd.

Pregnant albino BALB/c mice 30 ± 2 g, inbred in the Departmental Animal House, were divided into control and test groups and admitted into the study on delivery date designated day one. Test mouse dams received cimetidine 50 mg/kg i.p. daily from then onwards until pups were weaned naturally at the age of 6 weeks. Control mouse dams received appropriate volumes of normal saline. The pups themselves received neither cimetidine nor saline pretreatment. They were breastfed by the dams who had free access to standard mouse cubes (Pfizer Livestock Nigeria Ltd.) and water *ad libitum*. Each brood was made up of 6–8 pups and were caged with their mother in polypropylene cages measuring 40 × 25 × 15 cm. The cages were carpeted with wood shavings. Throughout the period of study (10 weeks), the animals were housed in a quiet room of ambient temperature 22 ± 2°, and alternating light and dark cycles of 12 hr each. The pups belonged to four groups: control male (CM), control female (CF), test male (TM) and test female (TF), depending on whether or not their mothers received normal saline or cimetidine. Both *in vivo* (pentobarbital sleeping time) and *in vitro* (aminopyrine *N*-demethylase assay) approaches were employed in assessing the level and activity of drug metabolizing enzymes.

Weighed two-week-old pups and their mothers from each of the groups were decapitated and the excised livers rinsed in 1.15% potassium chloride before weighing. 10% and 20% (w/v) homogenates in 0.25 M sucrose buffer pH 7.4 were prepared using pup and dam livers respectively. Differential centrifugation was used to prepare the 10,000 *g* post-mitochondrial supernatants which were employed in the assay of aminopyrine *N*-demethylase (APN-D) by following the formation of formaldehyde by the Nash reaction [10] as described by Holtzman *et al.* [11]. Protein was determined by the biuret method with BSA as standard [12]. Enzyme activities were expressed in nmol formaldehyde formed/mg protein/hr.

In a separate series of experiments the duration of sleep in response to pentobarbital sodium, pentobarbital sleeping time (PBST) using doses of 20, 30 and 40 mg/kg i.p. at 2, 3 and 4 to 10 weeks respectively, were determined for each group. The criterion for sleep was the loss of righting reflex. As in the *in vitro* experiments, only the test dams had received cimetidine 50 mg/kg i.p. daily for 6 weeks post partum.

Results and discussion

Control pups had only a small percentage (17.0–20.1%) of the APN-D activities of adult control dams. This lower drug metabolizing capacity of the pups was also reflected in the pups having a higher PBST (158–275%) compared to the dams, despite the former receiving a lower dose of pentobarbital sodium (50%). These results are in agreement with those of Jondorf *et al.* [13] who reported the inability of newborn mice to metabolize a wide range of drugs including aminopyrine and hexobarbitone. The present data also show that female pups have higher enzyme activity than their male counterparts. Again this is consistent with what had been shown in some strains of mice, although in other strains and most other animal species, the male has higher enzyme activity than the female [14].

The inhibitory effect of cimetidine on hepatic microsomal

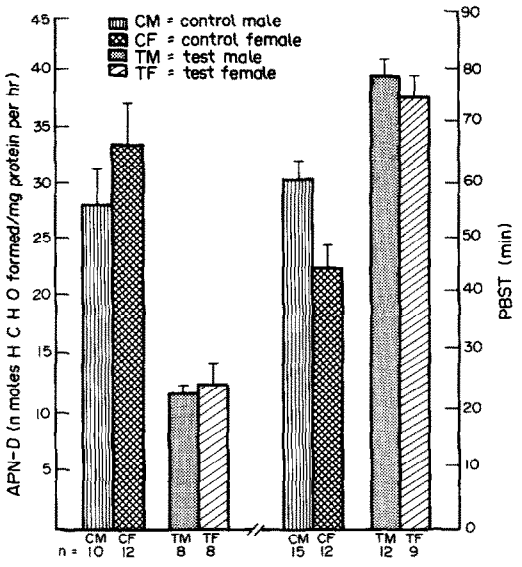

Fig. 1. Aminopyrine *N*-demethylase and pentobarbital sleeping time in control and test pups differentiated by sex. Bars represent means ± SEM. APN-D = Aminopyrine *N*-demethylase. PBST = Pentobarbital sleeping time.

enzymes have been studied by *in vitro* assay of APN-D and the *in vivo* determination of barbiturate sleeping time in control and pretreated groups [5, 7, 8]. In those reports, cimetidine pretreated groups showed reduced microsomal enzyme activity and prolonged sleeping time. The present data show that test pups as well as test dams exhibited similar trends, i.e. decreased APN-D activity and prolonged PBST when compared to their respective controls. Since only the dams received cimetidine, obviously, maternally administered cimetidine inhibited microsomal enzyme activity and therefore drug metabolism in mouse pups. The percentage decrease in APN-D activity was similar in male (58.4%) and female (62.1%) pups, whereas the percentage increase in PBST in male pups (28.7%) was less than half that in female pups (65.9%). Thus, this data show quantitative sex differences in the effect of cimetidine on drug metabolism in these pups, the females being more adversely affected.

In the control pups, the sex difference with respect to males was 18.6% for APN-D and 26.0% for PBST (Fig. 1). Since the effect of cimetidine in females was more than in males, the sex difference in the test pups was reduced to 8.2% for APN-D and 4.7% for PBST. Thus maternal cimetidine had the effect of reducing sex differences in enzyme activity in pups, encountered normally in this strain of mouse.

Neither liver/body weight ratio nor the 10,000 *g* supernatant protein was significantly altered by cimetidine pretreatment or by gender.

After establishing that *in vivo* PBST correlated with *in vitro* APN-D, subsequent development beyond two weeks of drug metabolizing capacity was followed using PBST as an index. PBST is inversely related to the level and activity of the mono-oxygenase system. From the age of 2 weeks to 10 weeks, control male pups slept consistently longer than control female pups indicating that the female had a higher ability to metabolize pentobarbital. Although this trend was present at all ages, it became significant from the age of 8 weeks after attainment of puberty [15]. Up to 8 weeks, the test pups slept longer than the control pups in both males and females (Fig. 2). This may be related to decreased ability of test pups to metabolize drugs due to the effect of cimetidine. However, while female pups were

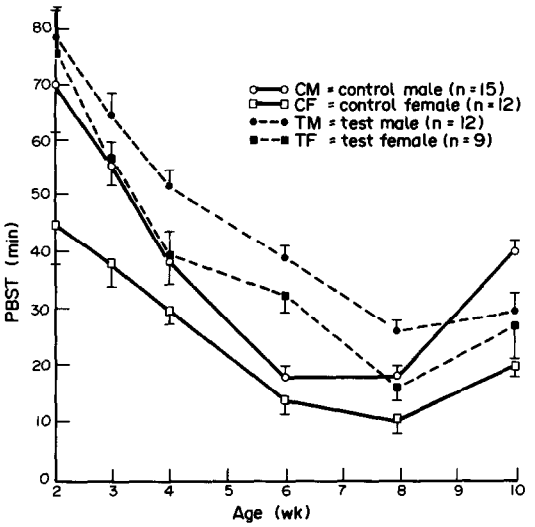

Fig. 2. PBST (min) in control and test pups at various ages differentiated by their sexes. The same animals (total 48) were studied throughout the period of 10 weeks. Each point refers to mean ± SE. Of the 24 comparisons made, the following were significant (ANOVA): CF/TF (2 week $P < 0.011$); CM/TM (4 week $P < 0.05$); CM/TM (6 week $P < 0.001$); CF/TF (6 week $P < 0.001$); CM/CF (8 week $P < 0.05$); CF/TF (8 week $P < 0.05$); CM/CF (10 week $P < 0.002$). PBST = Pentobarbital sleeping time.

affected as early as 2 weeks, male pups were affected from 4 weeks. Furthermore, the effect of cimetidine on male pups was no longer observed at an age of 8 weeks following cessation of maternal pretreatment and weaning 6 weeks.

On the other hand, the effect of cimetidine on female pups was still significant at ages of 6 and 8 weeks despite weaning and cessation of maternal pretreatment at 6 weeks of age. Thus the female pups were more adversely affected than the male. At 10 weeks, there was no significant difference between test and control pups for either sexes.

It is noteworthy that cimetidine-pretreated dams were not as adversely affected as the pups that received the drug indirectly in the breast milk, the per cent change in aminopyrine *N*-demethylase and pentobarbital sleeping time respectively being 19.1% and 24.6% for dams, 58.4% and 28.7% for male pups, 62.1% and 65.9% for female pups. According to Taylor *et al.* [16], cimetidine is excreted mostly in the urine, thus the higher susceptibility of the pups was significant since only a small fraction of the administered dose could have passed on to the pups in the milk. The effect of cimetidine in the pups may have been exaggerated because of the immature microsomal oxidation and renal excretory mechanisms in their development stages at this age.

In summary, maternally administered cimetidine during lactation inhibited the levels of drug metabolizing enzymes in mouse pups much more than it did in the dams which received the drug. Female pups were more adversely affected than the male. Obviously, the already poor ability of newborn mice to metabolize drugs was further jeopardized by maternal administration of cimetidine.

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