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DECREASED DURATION OF PENTOBARBITAL ANESTHESIA AND INCREASED PENTOBARBITAL METABOLISM IN IMMATURE RATS PERINATALLY EXPOSED TO CIMETIDINE

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Cimetidine, a histamine H_2-receptor antagonist, inhibits cytochrome P-450-mediated metabolism of most drugs and prolongs the duration of their actions as an untoward effect in adult humans and experimental animals [1-4]. The antihistamine is used in neonates for the prophylaxis of stress ulceration and management of gastrointestinal hemorrhage [10]; however, its effect on cytochrome P-450-mediated metabolism of drugs in the neonatal population has not been reported. In general, the duration of the effects of drugs is age-dependent and is usually longer in the immature of most species than in adults, in part because of the low levels and activities of cytochromes P-450 characteristic of the immature [5-7]. Qualitative differences in the hemoproteins may also exist between adults and the immature [7-9]. It is conceivable, therefore, that the effects of cimetidine on drug metabolism may differ qualitatively and quantitatively as a function of age. It is necessary to understand the drug interactions caused by cimetidine in the immature because newborns are subject to inadvertent as well as intentional exposure to multiple drugs. The present studies were undertaken to assess the effect of perinatal exposure to cimetidine on a pharmacodynamic response. The results show that the response variable - duration of pentobarbital-induced anesthesia (DPA) - was shortened in rats exposed neonatally to cimetidine. This effect is in contrast with a prolongation of the parameter in animals exposed to the antihistamine later in life.

MATERIALS AND METHODS

Chemicals and animals. Cimetidine, pentobarbital sodium, glucose-6-phosphate and NADP were purchased from the Sigma Chemical Co., St. Louis, MO. Glucose-6-phosphate dehydrogenase was purchased from Boehringer-Mannheim, St. Louis, MO. Pentobarbital sodium (ring-2-^{14}C, 21.8 mCi/mmol) was purchased from ICN Radiochemicals, Irvine, CA, and was further purified as described by Kuntzman *et al.* [11] prior to use. Timed pregnant Sprague-Dawley rats were purchased from Taconic Farms, Germantown, NY. They were housed, singly, in propylene cages containing Sanicel^R bedding (from Fisher Scientific Co.) under a 12-hr light-dark cycle,

22°C temperature, and 50% humidity. The animals were allowed free access to water and commercial Purina Rat Chow. All animals delivered on gestational day 21 ± 1 and each litter were reduced to a maximum of 10 pups at birth, keeping the ratio of males to females as close to 1 as possible. On postnatal day 1, 2 mothers received a single i.p. dose of cimetidine (10 mg/ml saline/kg) (CM-pretreated) while another two received appropriate i.p. doses of saline only (controls). Thereafter, CM-pretreated mothers received a 0.015% drinking solution of cimetidine in tap water. The latter was made fresh every other day and was stable for at least three days at room temperature as judged by chromatographic analysis [12]. All treatments of rats were terminated at weaning (postnatal day 21) and offspring were housed two to a cage, according to sex and pretreatment, in stainless steel hanging-type cages with perforated bottoms and were fed food and water ad lib.

Determination of duration of pentobarbital-induced anesthesia (DPA) and levels of pentobarbital in brain and plasma. Animals were injected with either ^{14}C-pentobarbital sodium (2 uCi/kg) or unlabelled pentobarbital at a dose of 35 mg/kg in saline. DPA was estimated as the time between the loss and return of the righting reflex. Immediately after regaining the righting reflex, rats that had received ^{14}C-pentobarbital were decapitated, and trunk blood and the entire brain were obtained from each animal. Unmetabolized ^{14}C-pentobarbital was extracted from aliquots of brain homogenates and plasma as described by Jacobson et al. [13] and quantified by scintillation spectrometry.

Preparation of liver microsomes and assay of the activities of drug-metabolizing enzymes. Washed microsomes from rats that had not received pentobarbital were prepared as described previously [8]. Microsomal oxidation of ^{14}C-pentobarbital was assayed as described by Jacobson et al. [13]. Microsomal cytochromes P-450 and b_5 were determined according to the method of Omura and Sato [14]. Protein was determined by the method of Lowry et al. [15], using bovine serum albumin as the standard.

Analysis of results. Differences between values for treated and control animals were analyzed by the Student's t test.

RESULTS AND DISCUSSION

Effect of cimetidine exposure on DPA. Weaned (28-day old) male and female offspring of CM-pretreated mothers exhibited decreased DPA as compared with their control counterparts; the decrease, however, was slightly more pronounced in females (48%) than in males (41%) (Table 1). Postnatal day 28 was chosen as a test age because rats, particularly females, metabolize pentobarbital maximally and are least sensitive to anesthesia from the barbiturate at that age [16]. Table 1 also shows that at 50 days of age, there appeared to be a slight shortening of DPA in female offspring of the CM-pretreated rats, but it was not statistically significant. The observed decrease in DPA in rats perinatally exposed to

cimetidine contrasts with the reported enhancement of the duration of action of most drugs in adult animals treated with the drug [17].

Table 1. Effect of perinatal cimetidine (CM) exposure on body weight, duration of pentobarbital-induced anesthesia (DPA), and some products of pentobarbital (PB) metabolism in rats

	Body Weight (g)	DPA (min)	Postanesthesia PB in: Plasma[b]	Brain[c]	Cytochrome P-450[#] (*)	Pentobarbital Metabolism[#] (**)
Control:						
Male (28)[a]	100 ± 10	114 ± 8	11	8	0.66 ± .05	6.2 ± .40
(50)[a]	272 ± 45	35 ± 4	ND	ND	ND	ND
Female (28)[a]	100 ± 10	128 ± 17	9	10	0.50 ± .06	4.2 ± .20
(50)[a]	190 ± 11	203 ± 29	ND	ND	ND	ND
CM-pretreated:						
Male (28)[a]	120 ± 13	67 ± 13[d]	9	10	0.87 ± .13	7.7 ± .40
(50)[a]	259 ± 21	44 ± 10	ND	ND	ND	ND
Female (28)[a]	112 ± 10	66 ± 4[e]	8	11	0.77 ± .06[f]	5.6 ± .10[f]
(50)[a]	208 ± 14	165 ± 17	ND	ND	ND	ND

Unless indicated otherwise, each value is the mean (± S.D.) from 5 rats, all from a total of 2 litters. * Content (nmole/mg hepatic microsomal protein). ** nmole Total water-soluble products formed/mg hepatic microsomal protein/min. # Rats used for these assays did not receive pentobarbital. ND = not determined. [a] Age (days). [b] μg/ml Plasma; N = 2 - 3 rats from 2 litters. [c] μg/g Whole brain; N = 2 - 3 rats from 2 litters. [d] Significantly different from control males ($P < 0.0005$). [e] Significantly different from control females ($P < 0.005$). [f] Significantly different from control females ($P < 0.05$).

To assess whether the cimetidine-induced attenuation of DPA resulted from tolerance to pentobarbital or from enhanced metabolism of the barbiturate, post-anesthesia brain and plasma levels of pentobarbital were compared in offspring of control and CM-pretreated rats. As shown in Table 1, these variables were identical in the two groups, suggesting that enhanced metabolism was a major cause of the decreased DPA in offspring of CM-pretreated rats.

Table 2. Effect of cimetidine regimen on the duration of pentobarbital-induced anesthesia in 28-day old rats

Treatment Regimen	Duration of anesthesia (min)	Cytochrome P-450[a]
Control:		
Male	114 ± 8	0.66 ± .05
Female	128 ± 17	0.50 ± .06
+ Cimetidine (Postnatal Day 28)*:		
Male	280 ± 48[b]	ND
Female	310 ± 31[d]	ND
+ Cimetidine (Postnatal Day 22 - 28)**:		
Male	248 ± 32[b]	0.57 ± .05
Female	285 ± 29[d]	0.44 ± .06
Cimetidine-pretreated (Postnatal Day 1-21)***:		
Male	67 ± 13[b]	0.87 ± .13
Female	66 ± 4[d]	0.77 ± .05[e]
+ Cimetidine (Postnatal Day 28)*:		
Male	142 ± 12[c]	ND
Female	185 ± 22[e]	ND

Each value is the mean (± S.D) of 3 - 5 rats. * 10 mg Cimetidine/kg was administered i.p. 30 min prior to the administration of pentobarbital. ** 10 mg/kg i.p. daily for the duration indicated. *** Treatment was as described in Materials and Methods. ND = not determined. [a] nmole/mg Microsomal protein.

[b] $P < 0.0005$, [c] $P < 0.05$: Levels of difference between treated and control males.

[d] $P < 0.0005$, [e] $P < 0.05$: Levels of difference between treated and control females.

Effect of perinatal cimetidine exposure on hepatic microsomal pentobarbital metabolism and cytochromes P-450 content. Perinatal cimetidine exposure was also associated with increased cytochromes P-450 content and pentobarbital oxidation in hepatic microsomes from weaned rats; this effect was statistically significant only in weaned female 28-day old rats (Table 1). In contrast with the findings of the present study, neither xenobiotic metabolism nor cytochrome P-450 was induced in adult male rats pretreated with the antihistamine [4]. However, it was observed in the present study (Table 2) that treatment of weaned offspring of control or CM-pretreated rats with single or multiple doses of cimetidine only prolonged

rather than shortened DPA, and did not induce hepatic microsomal cytochromes P-450; this result corroborates the reported failure of the drug to induce drug metabolism in adults [4]. Nutritional deficits, which have been reported to induce the metabolism of some drugs [17], were not a significant factor in the present observations as evidenced by the failure of perinatal cimetidine exposure to affect body weight of the animals (Table 1).

From the foregoing results, it is suggested that the effect of cimetidine on the induction of cytochrome P-450 and the rate of drug metabolism may be peculiar to perinatal exposure to the drug and that inhibition may be the characteristic response in the adult. Cimetidine may have induced non-cytochromes P-450 enzymes that could have contributed to the observed decrease in DPA. However, DPA is determined primarily by cytochrome P-450-mediated metabolism of the barbiturate [5,6,16]. Events or agents resulting from the actions of cimetidine, rather than the antihistamine *per se* may have caused the induction of the hemoprotein in the present studies. Likely direct inducers include endogenous hormones, such as androgens. Testosterone, an androgen, induces hepatic cytochromes P-450 [20,21] and cimetidine increases the systemic level of the androgen [18], perhaps, by inhibiting the cytochrome P-450-mediated hepatic oxidation of the androgen [19].

In summary, data are presented that exposure of perinatal rats to cimetidine shortens DPA and increases the rate of pentobarbital metabolism and cytochromes P-450 content in hepatic microsomes. Postweaning exposure to the antihistamine, on the other hand, increases DPA. It is likely that these effects are caused by hormonal alterations effected by cimetidine in the perinatal rat. Since humans and rodents respond similarly to cimetidine, the possibility of increased metabolism of endogenous compounds and decreased efficacy of concomitantly administered drugs in neonates receiving cimetidine deserves consideration. The involvement of androgens or other hormones in the observed cimetidine-induced increase in drug metabolism and the cytochrome P-450 species involved is being investigated.

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