Table 1. Effect of an acute soman dose on the plasma levels of corticosterone, ACTH and β-endorphin*

Treatment	Average toxic sign score	Corticosterone (ng/ml)	ACTH (pg/ml)	β-Endorphin (pmol/L)
Saline, non-stressed	—	111 ± 29	85 ± 5	27 ± 7
Soman, 80 μg/kg	3.1	410 ± 21†	586 ± 105‡	115 ± 7†
Saline, stress	—	489 ± 20†	379 ± 45†	108 ± 5†

* Blood sample was obtained 18 min post-treatment. Values are means ± SE, N = 8.
† Significantly different ($P < 0.05$) from saline-nonstressed group.
‡ Significantly different ($P < 0.05$) from both groups.

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Differential effects of cimetidine, ranitidine and famotidine on the hepatic metabolism of estrogen and testosterone in male rats

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Cimetidine (*N*″-cyano-*N*-methyl-*N*′-[2[[(5-methyl-1*H*-imidizol-4-yl)methyl]thio]-ethyl]-guanidine) is a histamine H_2-receptor antagonist which contains an imadazole ring and binds, as a type II ligand, to cytochrome P-450 [1–5]. Such interactions lead to inhibition of cytochrome P-450 function and decreased metabolism of exogenous pharmacological

agents such as antipyrine [6–8], trimethadione [9], 7-ethoxycoumarin [1, 10], hexabarbital and theophylline [2, 11], and aniline [12]. Recently, we reported that cimetidine also alters the hepatic metabolism of estrogens by decreasing estradiol 2-hydroxylation [13], indicating that cimetidine affects the biotransformation of both endogenous and exogenous compounds.

Ranitidine (*N*[2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]thio]ethyl] - *N'* - methyl - 2 - nitro - 1,1 - ethenediamine) and famotidine (3-[[[2-[(amino-iminomethyl)amino]-4-thiazolyl]methyl]-thio]-*N*-(amino-sulfonyl) propanimidamide) are two second generation H_2-receptor antagonists which lack imidazole rings and have little or no effect on the metabolism of exogenous pharmaceutical agents [4, 14–19]. The aim of the present study was to compare the effects of these three H_2-receptor antagonists on estrogen hydroxylation and, in view of the recent demonstrations that cimetidine impairs testosterone hydroxylation in mouse hepatic microsomes [20, 21], to determine if testosterone metabolism followed similar patterns in male rats.

Materials and methods

Sterile injectable solutions of cimetidine (Tagamet), ranitidine (Zantac) and famotidine (Pepcid) were purchased, respectively, from Smith, Kline & Beckman (Philadelphia, PA); Glaxo (Research Triangle Park, NC); and Merck, Sharpe & Dohme (Rahway, NJ). [2-^{3}H]Estradiol (22.7 Ci/mmol), [16α-^{3}H]estrone (15–30 Ci/mmol) and [4-^{14}C]testosterone (50 mCi/mmol) were purchased from New England Nuclear (Boston, MA). All other reagents were of the highest grade commercially available.

Adult male Sprague–Dawley rats (mycoplasma free: 200–250 g) were purchased from Charles River (Wilmington, DE) and housed in The Rockefeller University Laboratory Animal Research Center at 23 ± 1° in 12-hr light cycled rooms (lights on 7:00 a.m.) with Purina rat chow and water *ad lib.* Animals were acclimatized for 1 week, and then injected intraperitoneally with saline or various concentrations of the three H_2-receptor antagonists. Rats were treated at 8:00 a.m. and 5:00 p.m. for 2 days; on the third day, 1 hr after the fifth and last injection, animals were killed by decapitation. Livers were perfused *in situ* with 30 ml of ice-cold saline, then excised, weighed and homogenized. Differential centrifugation was utilized to prepare mitochondrial and microsomal fractions as previously described [22]. Estrogen [23] and testosterone hydroxylation [24], and heme pathway and cytochrome P-450 contents and functions [13] were determined as described previously. Spectral assays were performed on an Aminco Chance DW2A scanning spectrophotometer and fluorometric assays on an Hitachi MPFIV fluorescence spectrophotometer with an R928 photomultiplier tube. Protein content was determined by the method of Lowry *et al.* [25] using bovine serum albumin as a standard. Significance of differences between means was analyzed by Student's *t*-test, ANOVA and Dunnett's multiple comparison test utilizing The Rockefeller University Hospital Clinfo System.

Results

The effects of the three H_2 receptor antagonists on the metabolism of estrogen are compared in Table 1. Estradiol 2- and 16α-hydroxylation were decreased significantly following cimetidine (15 mg/100 g body wt × 5), confirming our earlier observations [13]. Neither ranitidine (2.5 mg/100 g body wt × 5) nor famotidine (0.5 mg/100 g body wt × 5) had any discernible effect on either 2- or 16α-hydroxylation of estradiol. Cimetidine also decreased the formation of polar metabolites of testosterone and produced a marked increase in 3-androstanediol, the fully reduced form of this androgen; ranitidine and famotidine were without effect (Table 2). The doses of H_2-receptor antagonists utilized were scaled down from the starting cimetidine concentration of 15 mg/100 g body wt relative to commonly used clinical dosages. Standard oral clinical dose regimens are 300 mg every 6 hr for cimetidine, 50 mg every 6 hr for ranitidine and 10 mg every 6 hr for famotidine (i.e. the dosage ratio cimetidine : ranitidine : famotidine is 30:5:1). To determine if the lack of inhibitory effect of ranitidine on estradiol metabolism was due simply to the lower concentration of drug utilized as compared to cimetidine, doses of 2.5, 5 and 15 mg/100 g body wt of ranitidine were compared; none of these doses produced any decrease in 2 or 16α-hydroxylation (data not shown).

In contrast to our previous results with cimetidine [13], there was no change in the hepatic contents of cytochrome P-450 or in the activities of δ-aminolevulinic acid synthase, heme oxygenase, aryl hydrocarbon hydroxylase, 7-ethoxycoumarin de-ethylase, alanine hydroxylase or ethyl morphine demethylase following treatment with these doses of ranitidine or famotidine; nor did ranitidine alter significantly the K_m or V_{max} of hepatic estradiol 2-hydroxylase (data not shown).

Discussion

In this paper, we have shown that treatment of male rats with cimetidine, a commonly prescribed H_2 histamine receptor antagonist which decreases 2- and 16α-hydroxylation of estradiol by liver microsomes [13], also inhibited androgen metabolism. Administration of cimetidine diminished the formation of polar metabolites (hydroxylated

Table 1. Effects of H_2-receptor antagonists on estrogen metabolism in male rats

Treatment	[2-^{3}H]E_2		[16α-^{3}H]E_2	
	% ^3H_2O	% WSP	% ^3H_2O	% WSP
Saline	32.5 ± 1.80	41.9 ± 2.30	4.8 ± 0.53	20.9 ± 1.18
Cimetidine 15 mg/100 g body wt × 5	12.2 ± 1.57*	19.9 ± 1.56*	2.0 ± 0.30*	9.6 ± 1.02*
Ranitidine 2.5 mg/100 g body wt × 5	37.06 ± 3.65	45.7 ± 3.65	5.6 ± 0.66	23.4 ± 1.45
Famotidine 0.5 mg/100 g body wt × 5	33.2 ± 1.91	40.6 ± 2.07	5.2 ± 0.38	20.8 ± 1.51

Adult male rats were treated i.p. with the indicated doses of three H_2-receptor antagonists. After five doses, the animals were killed, and the hepatic microsomal metabolism of [2-^{3}H]E_2 and [16α-^{3}H]E_2 was determined as described in Materials and Methods. Means ± SE of eight to nine determinations from four rats per group are presented. WSP = water-soluble products.

* $P < 0.05$ vs control (Dunnett's).

Table 2. Effects of H_2-receptor antagonists on testosterone metabolism in male rats

Treatment	% WSP	% Polar metabolites	% Adiol
Saline	14.1 ± 1.1	42.6 ± 3.1	6.5 ± 1.7
Cimetidine			
15 mg/100 g body wt × 5	10.1 ± 0.1*	25.0 ± 3.1*	26.9 ± 2.7†
Ranitidine			
2.5 mg/100 g body wt × 5	14.7 ± 1.5	40.4 ± 2.6	7.3 ± 3.9
Famotidine	12.2	38.1	13.0
0.5 mg/100 g body wt × 5	(11.6–12.9)	(31.7–44.6)	(11.0–15.0)

Adult male rats were treated as in Table 1, and the hepatic microsomal metabolism of [4-^{14}C]testosterone was determined as described in Materials and Methods. Means ± SE of two determinations from four rats per group are presented. For famotidine, the values are averages from two animals (range given). WSP = water-soluble products; Adiol = 3-androstanediol.

* $P < 0.02$ vs respective saline control (*t*-test).

† $P < 0.001$ vs respective saline control (*t*-test).

derivatives) of testosterone and increased the yield of 3-androstanediol, the fully reduced steroid. The identity of this last compound was determined as described previously [24] and is likely due to compensatory reductive metabolism of intermediates which are normally hydroxylated in control animals.

Cimetidine has been shown previously to decrease the 6β-, 7α- and 16α-hydroxylation of testosterone by mouse liver microsomes, an effect not seen with ranitidine or famotidine which do not contain an imidazole ring structure [20, 21]. In addition, famotidine, unlike cimetidine, does not alter the oxidative metabolism of cortisol in humans [26]. Cimetidine is known to possess weak antiandrogenic activity and bind to the androgen receptor without eliciting an androgenic response [27]; it is conceivable that these latter effects could modulate the hepatic metabolism of testosterone, but this is unlikely in view of the short-term treatments used in our studies.

Unlike cimetidine, neither ranitidine nor famotidine affected the oxidative metabolism of estradiol and this was found to be true for several other cytochrome P-450-catalyzed reactions which we have shown previously are decreased by cimetidine [13]. Increasing the dose of ranitidine (to the same concentration as that used in experiments with cimetidine) did not result in changed rates of 2- or 16α-hydroxylation of estradiol and this drug had no significant effect on the kinetics of these reactions.

In summary, cimetidine administered to male rats at standard clinical doses altered the hepatic metabolism of both estradiol and testosterone whereas ranitidine and famotidine, under similar conditions, were completely without effect.

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