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THE EFFECT OF CIMETIDINE ON *IN VITRO* AND *IN VIVO* MICROSOMAL DRUG METABOLISM IN THE RAT

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Abstract—The effect of cimetidine on rat liver microsomal drug metabolism *in vitro* and *in vivo* was studied. Cimetidine inhibits aminopyrine *N*-demethylation and benzo[*a*]pyrene hydroxylation in a non-competitive manner with inhibition constants between 1 and 10 mM. Benzo[*a*]pyrene hydroxylation in liver microsomes from 3-methylcholanthrene-pretreated rats is not appreciably inhibited by cimetidine indicating some specificity in terms of different cytochrome P-450 forms. Cimetidine gives rise to a type II spectral change with a spectral dissociation constant of about 0.1 mM. The prolonged administration of cimetidine does not result in the induction of hepatic drug metabolism. Pretreatment of rats with cimetidine prolongs aminopyrine half-life and hexobarbital sleeping time. These results demonstrate that cimetidine is an *in vitro* inhibitor of microsomal drug metabolism in the rat and this inhibition leads to pharmacokinetic drug–drug interactions *in vivo*.

Cimetidine, an antagonist of histamine H_2-receptors, inhibits gastric acid secretion and is used in peptic ulcer disease [1]. Cimetidine is excreted largely unchanged in the urine, but a small part is metabolized, the sulphoxide being the major metabolite in man, the dog and the rat [2]. Sulphoxidation is catalysed by the microsomal cytochrome P-450-linked monooxygenase system [3] and interactions with other microsomally metabolized drugs may theoretically be possible. Early studies revealed no significant interactions [4], but recent findings are beginning to accumulate indications that it may interfere with the action of other compounds. The reports of Flind [5] and Silver and Bell [6] showed that cimetidine potentiates the hypoprothrombinemic effect of warfarin. Our own preliminary study in the rat [7] demonstrated that cimetidine inhibits the *in vitro* microsomal metabolism of aminopyrine and benzo[*a*]pyrene, prolongs aminopyrine half-life and potentiates hexobarbital sleeping time. Recently we have also shown that cimetidine prolongs significantly the half-life of antipyrine in human volunteers and inhibits the activities of microsomal drug-metabolizing enzymes in liver biopsy material [8]. Serlin *et al.* [9] demonstrated that cimetidine reduces the clearance of warfarin and antipyrine, and increases the prothrombin time and the plasma warfarin concentration in man. All these studies taken together suggest that cimetidine has significant interactions with other drugs and that the apparent mechanism is inhibition at the level of microsomal metabolism. After submitting the present paper for publication we became aware of the study of Rendić *et al.* [10], which demonstrated that cimetidine interacts *in vitro* with rat liver microsomes and inhibits the *O*-dealkylation of 7-ethoxycoumarin.

The purpose of the present study was to investigate in more detail the interactions and effects of cimetidine in relation to microsomal drug metabolism.

MATERIALS AND METHODS

Animals. Adult male rats of the Sprague–Dawley strain weighing between 200 and 250 grams were used. The animals were maintained under regularly alternating periods of light and dark and had free access to food (standard rat diet from Hankkija Oy, Turku, Finland) and water. All the animals were housed in polyester cages, 4–6 in each cage, at 20–23° and 40–60 per cent humidity. Pine wood shavings were used on the bottom of the cages. The animals were killed by decapitation.

Preparation of tissue fractions. All subsequent procedures were carried out in a cold room at +4°. The livers were rinsed in ice-cold buffer, blotted dry, weighed, cut into small pieces and homogenized with a Potter–Elvehjem glass–teflon homogenizer in 4 volumes of 0.1 M sodium/potassium phosphate buffer, pH 7.4. The homogenate was centrifuged at 12,000 *g* and 100,000 *g* for 20 and 60 min respectively. The microsomes were re-suspended in phosphate buffer to give a final protein concentration of about 15 mg/ml.

Enzyme assay. Aminopyrine *N*-demethylase, benzo[*a*]pyrene hydroxylase, and 7-ethoxycoumarin *O*-deethylase were determined in a cofactor mixture with final concentrations of KCl 50 μmoles, $MgCl_2$ 2.5 μmoles, glucose-6-phosphate 3 μmoles, glucose-6-phosphate dehydrogenase 10 units, NADP 0.5 μmoles, enzyme preparation (microsomal protein about 0.5 mg for benzo[*a*]pyrene monooxygenase and 7-ethoxycoumarin *O*-deethylase and about 1–2 mg for aminopyrine *N*-demethylase), substrate (aminopyrine 2.5 μmoles in water; benzo[*a*]pyrene 80 nmoles in acetone and 7-ethoxycoumarin 500 nmoles in 50% ethanol) and 50 mM sodium/potassium phosphate buffer, pH 7.4, added to a final volume of 1 ml. Cimetidine was added in 10 μl of dimethylsulphoxide. Incubation was started

with enzyme preparation (aminopyrine *N*-demethylase) or the substrate (benzo[*a*]pyrene hydroxylase and 7-ethoxycoumarin *O*-deethylase) after a 2-min preincubation period and took place in a metabolic shaker under atmospheric air at +37°. Incubation lasted 15 min (benzo[*a*]pyrene and 7-ethoxycoumarin) or 20 min (aminopyrine), linearity being maintained for this time. All determinations were carried out in duplicate. The formation of formaldehyde from aminopyrine was measured by the method of Nash [11] as described by Cochin and Axelrod [12]. The formation of fluorescent phenolic benzo[*a*]pyrene metabolites was determined according to Nebert and Gelboin [13]. The fluorescence of 7-hydroxycoumarin was measured according to Greenlee and Poland [14].

Cytochrome P-450 was determined according to Omura and Sato [15], and protein was measured by the Lowry method [16].

Spectral interactions. For the determination of spectral interactions, the microsomal suspension was diluted with phosphate buffer to yield a protein concentration of about 1.5–2 mg/ml. All spectra were measured at room temperature in 1 cm cells in a Shimazu model MPS-50L recording spectrophotometer. Baselines were traced. Difference spectra were obtained by the addition of microliter quantities of a solution of cimetidine in dimethylsulphoxide to one of the cuvettes, while an equal volume of the solvent was added to the reference cuvette. The spectra were traced within a couple of minutes of the addition of the compound. The extent of the spectral change was measured from the difference between the wavelengths of minimum and maximum absorption (about 393 and 430 mm respectively).

Elimination of [^{14}C]aminopyrine. Male Sprague–Dawley rats were anaesthetized with urethane, 1.2 g/kg i.p., and the trachea and femoral artery cannulated. The rats were allowed to recover from the surgery for about 30 min before an intraperitoneal injection of 40 mg/kg of cimetidine (in saline) or saline alone (controls). [^{14}C]Aminopyrine (8.2 mCi/mmole) was injected into the tail vein 30 min later and blood samples were drawn from the femoral artery after 15, 30, 60 and 120 min. Blood (0.5 ml) was diluted with 0.8 ml of heparine–saline (150 units/ml of 0.9% NaCl) and centrifuged for 2 min at 8000 *g*. [^{14}C]Aminopyrine was extracted from 1 ml of the supernatant by the method of Brodie and Axelrod [17]. The recovery of [^{14}C]Aminopyrine was 78 per cent (n = 4, range 73–82%).

Hexobarbital sleeping time. Hexobarbital sleeping time was studied in male Sprague–Dawley rats of weight 200–250 g. Hexobarbital sodium (100 mg/kg) was injected intraperitoneally 30 min after an intraperitoneal injection of cimetidine (in saline) or saline (controls).

Chronic treatment with cimetidine. Male Sprague–Dawley rats (initial weight 200–250 g) were used. Cimetidine (75 mg/kg) was injected subcutaneously three times daily for 6 days. The cimetidine solution (22.5 mg/ml) consisted of 1.35 g of cimetidine, 6.6 ml of 1 N HCl, 12 ml of 0.1 N NaOH and distilled water added to 60 ml. The control rats received an equal volume (0.33 ml/100 g) of 100 mM NaCl, pH 6.0, corresponding approximately to the pH and osmolarity of the cimetidine solution. The animals were decapitated 24 hr after the last injection and microsomes were prepared as described above.

RESULTS

Effect of cimetidine on microsomal monooxygenase activities. Figure 1 shows that cimetidine inhibits

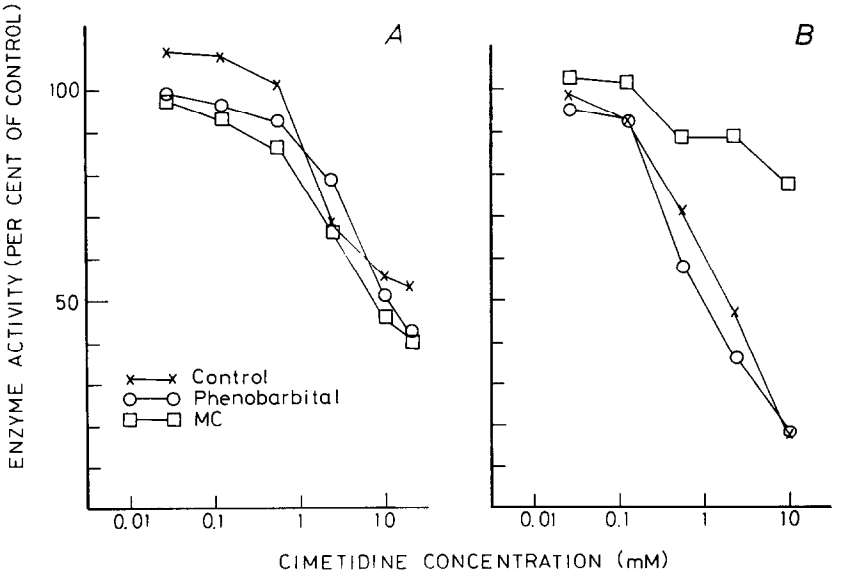

Fig. 1. Inhibition by cimetidine of aminopyrine *N*-demethylation (A) and benzo[*a*]pyrene hydroxylation (B) catalysed by rat liver microsomes *in vitro*. Each inhibition curve is the mean of experiments with four different rat liver microsomal preparations. Standard deviations for different data points were between 1 and 8 per cent.

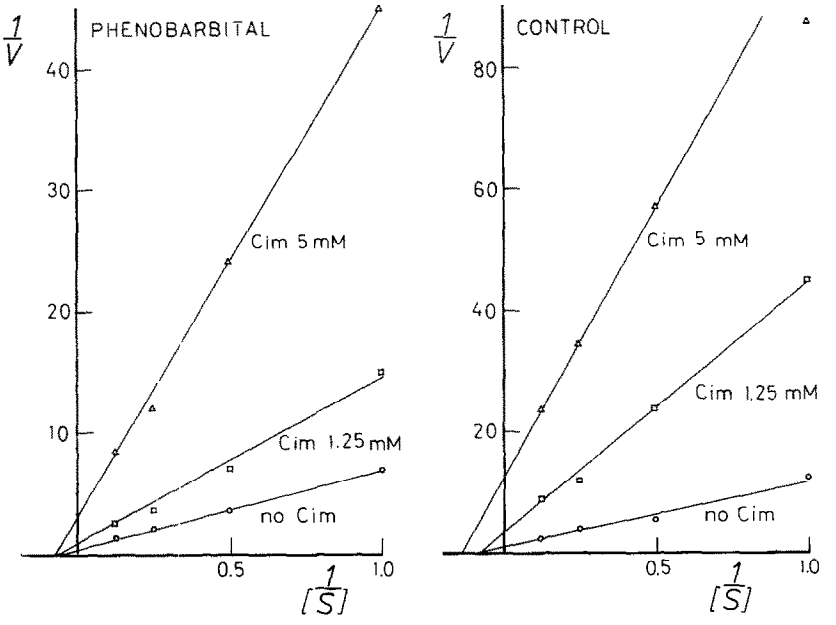

Fig. 2. Double-reciprocal plots of the inhibition of benzo[*a*]pyrene hydroxylation catalysed by rat liver microsomes from control and phenobarbital-pretreated animals.

both aminopyrine *N*-demethylase and benzol[*a*] pyrene hydroxylase activities, although to different extents. The qualitative difference involves the relative inability of cimetidine to inhibit benzo[*a*]pyrene monooxygenase in microsomes from MC-pretreated rats, whereas in microsomes from control and phenobarbital-pretreated rats inhibition of benzo[*a*]-pyrene monooxygenase was attained at concentrations of about 1 mM cimetidine. In the case of aminopyrine *N*-demethylase activity, cimetidine inhibited this activity in the microsomes to comparable extents regardless of the pretreatment given. A 50 per cent inhibition of aminopyrine *N*-demethylase was attained with a cimetidine concentration approaching 10 mM.

Cimetidine also inhibits coumarin 7-hydroxylase and ethoxycoumarin *O*-deethylase activities (data not illustrated).

Mode of inhibition of benzo[a]*pyrene monooxygenase by cimetidine.* Figure 2 shows the double-reciprocal plots of benzo[*a*]pyrene monooxygenase activity in the presence of cimetidine. The mode of inhibition is interpreted as being non-competitive, since the K_m value for benzo[*a*]pyrene does not change as a function of cimetidine concentration. Similar results were obtained with respect to aminopyrine *N*-demethylation (data not illustrated).

Interactions of cimetidine in vitro *with cytochrome P-450 after various pretreatments.* Addition of cimetidine to the microsomes produced a characteristic spectrum with a trough at about 393 nm and a peak at about 430 nm (Fig. 3). This difference spectrum is usually referred to as the type II spectrum [18]. All microsomes, whether obtained from the control, phenobarbital-pretreated or MC-pretreated rats, gave qualitatively similar spectra with troughs and peaks at approximately similar wavelengths. Eadie–Hofstee analysis (Fig. 4) showed that the K_d values do not differ very much and are all in the range between 110 and 130 μM. Only with MC-microsomes was there some indication of a two-phase plot with a higher K_d value. Relating the magnitude of the difference spectrum to the cytochrome P-450 content, the microsomes from the MC-pretreated rats gave higher spectral changes than those from either the control or phenobarbital-pretreated rats.

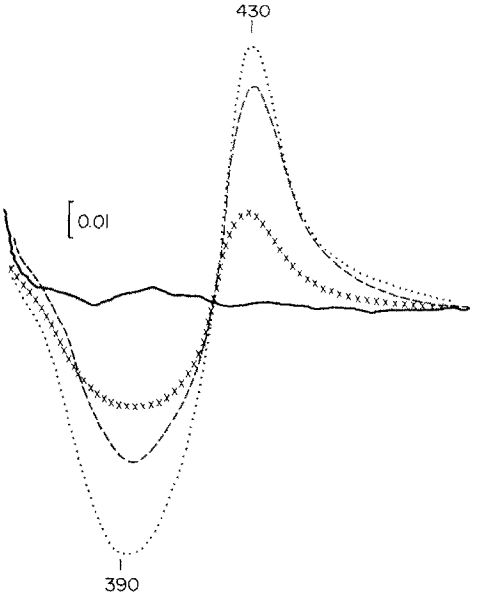

Fig. 3. Spectral changes induced by cimetidine with liver microsomes from control(xxxxxx), phenobarbital-pretreated (– – – – –) and 3-methylcholanthrene-pretreated rats (●●●●●). Cytochrome P-450 per ml of solution were 3.20, 1.76 and 0.92 nmoles for phenobarbital 3-MC and control microsomes respectively.

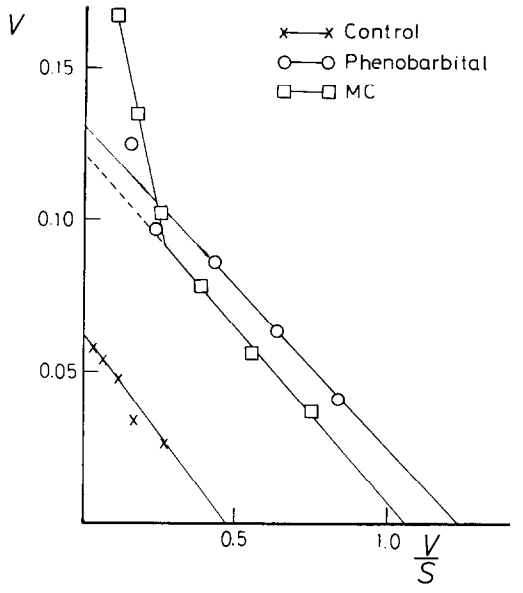

Fig. 4. Eadie–Hofstee analysis of the interactions of cimetidine with liver microsomes from control, phenobarbital-pretreated and 3-methycholanthrene-pretreated rats. Amounts of cytochrome P-450 per ml of solution were 2.95, 1.54 and 0.90 nmoles for phenobarbital 3-MC and control microsomes respectively. The concentration of cimetidine varied between 0.05 and 4 mM.

Effect of in vivo *administration of cimetidine on monooxygenase activities.* The effect of cimetidine administered three times a day at a dose level of 75 mg/kg body weight for six days on monooxygenase activities in the rat is shown in Table 1. The weight gain was marginally retarded and there were no changes in cytochrome P-450 content, microsomal protein, 7-ethoxycoumrain *O*-deethylase or aminopyrine *N*-demethylase activities. Benzo[*a*]pyrene hydroxylation was significantly higher in the control group.

Effect of cimetidine on plasma elimination of aminopyrine. Figure 5 demonstrates the effect of cimetidine on the plasma elimination of aminopyrine. Cimetidine prolonged the plasma half-life from 37 to 83 min, and also affected the volume of distribution of [^{14}C]aminopyrine, the V_d value for the controls being 1.34 ± 0.25 (S.D.) and that for the cimetidine-treated rats 0.82 ± 0.16 (S.D.). The difference is highly significant ($P < 0.005$).

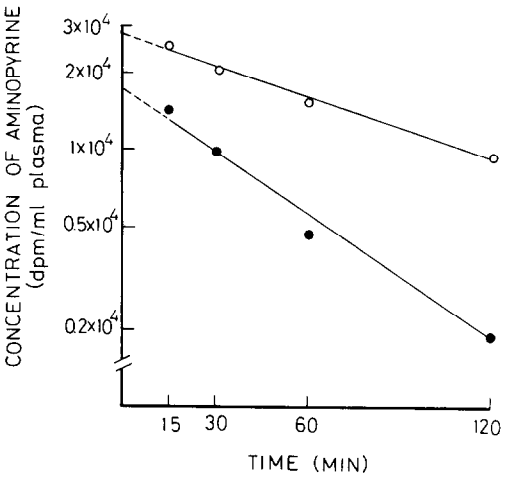

Fig. 5. Effect of cimetidine on the elimination of aminopyrine from plasma. 10 μCi/kg of [^{14}C]aminopyrine was injected i.v. 30 min after the i.p. injection of cimetidine (40 mg/kg) (○——○) or saline (controls, ●——●).

Effect of cimetidine on hexobarbital sleeping time. Treatment of rats with 40 and 80 mg/kg of cimetidine prolonged hexobarbital-induced sleeping time from 10 to 27 and 67 min respectively (Fig. 6). Hexobarbital concentrations on awakening were not determined.

DISCUSSION

A number of clinical studies have demonstrated interactions between cimetidine and oral anticoagulants [5, 6, 9, 19]. Our preliminary studies in the rat

Table 1. The effect of *in vivo* administration of cimetidine on body weight, liver weight and hepatic monooxygenase activities in the rat*

Parameter	Control	Cimetidine-pretreated	Significance of difference
Body weight at the end of treatment (g)	298 ± 10	282 ± 13	NS
Liver weight (g)	13.0 ± 1.0	12.2 ± 0.6	NS
Microsomal protein (mg/g)	12.7 ± 1.3	13.7 ± 1.1	NS
Cytochrome P-450 (nmoles/mg microsomal protein)	0.77 ± 0.15	0.81 ± 0.10	NS
Benzo[*a*]pyrene hydroxylase (pmoles/mg protein/min)	51.0 ± 13.3	32.2 ± 9.6	$P < 0.01$
7-Ethoxycoumarin *O*-deethylase (pmoles/mg protein/min)	212 ± 23	206 ± 16	NS
Aminopyrine *N*-demethylase (nmoles/mg protein/min)	0.81 ± 0.10	0.84 ± 0.14	NS

* A group of 7 rats was treated three times daily with s.c. injections of cimetidine (75 mg/kg body wt) for 7 days. Another group of 7 rats served as controls and were treated with 100 mM NaCl, pH 6.

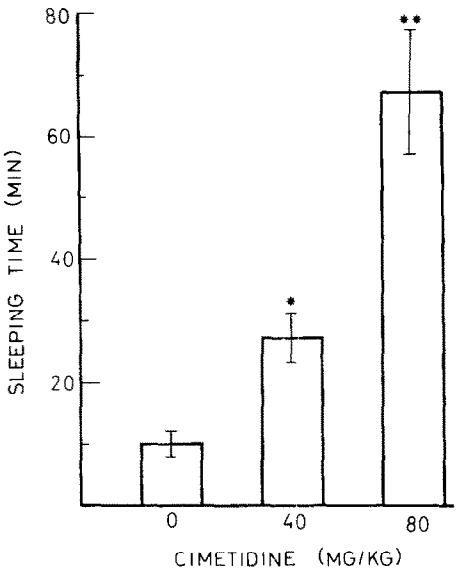

Fig. 6. Effect of cimetidine on hexobarbital sleeping time. Hexobarbital sodium (100 mg/kg) was injected i.p. 30 min after the i.p. injection of cimetidine. Values are means standard errors of the mean from six animals (*, $P < 0.01$; **, $P < 0.001$).

[7] and in man [8] and those of other groups in man [9, 20] strongly imply that interactions observed *in vivo* are due to the inhibition of the metabolism of the compounds studied by cimetidine. The present results similarly show clearly that cimetidine inhibits the microsomal metabolism of aminopyrine and benzo[*a*]pyrene, interacts with cytochrome P-450 giving rise to a characteristic difference spectrum, and prolongs both aminopyrine half-life and hexobarbital sleeping time. On the other hand, prolonged administration of cimetidine does not induce microsomal drug metabolism.

Unlike some other imidazole compounds [21], cimetidine is not a potent inhibitor of microsomal drug metabolism. The inhibitor/substrate ratios for 50 per cent inhibition for aminopyrine and benzo[*a*]pyrene are about 2 and 40 respectively, and the approximate K_i values for cimetidine range from about 1 to 10 mM. Furthermore, it is of interest that cimetidine inhibits benzo[*a*]pyrene hydroxylase activity in microsomes from 3-methylcholanthrene-induced rats only very weakly. It has been demonstrated that benzo[*a*]pyrene hydroxylase activity in 3-methylcholanthrene-pretreated animals is associated with a different cytochrome P-450 from that in control or phenobarbital-pretreated animals [22]. Aminopyrine is not readily *N*-demethylated by this variant cytochrome [23]. It thus seems that the inhibitory action of cimetidine has some specificity in terms of cytochrome P-450.

Usually substrates and inhibitors of microsomal monooxygenase give rise to typical difference spectra, and cimetidine is not an exception. It gives rise to the type II spectral change, with a characteristic trough at 390 nm and a peak at 430 nm. The spectral dissociation constants observed are much lower than the inhibition constants. K_d values are in the range of 0.1–0.2 mM, whereas the K_i values range from 1 to 10 mM, a difference of one to two orders of magnitude. Furthermore, the spectral change calculated per unit of cytochrome P-450 is largest with microsomes from 3-methylcholanthrene-pretreated rats, whereas benzo[*a*]pyrene hydroxylation, which is catalysed most efficiently by these microsomes, is hardly inhibited by cimetidine.

It seems that relationships between inhibitory properties and spectral interactions are not straightforward and great care must be exercized in the interpretation of the results. Other investigators have discussed in great detail the complexity of microsomal enzyme inhibition by compounds with type I and II interaction spectra [24, 25]. The alternative substrate concept of inhibition requires that the inhibition must be competitive and that the inhibition constant must not differ from the Michaelis constant for the metabolism of the inhibitor [24]. The inhibitory effect of cimetidine on benzo[*a*]pyrene hydroxylation and aminopyrine *N*-demethylation is clearly non-competitive, as shown here, but competitive, with respect to *O*-dealkylation of 7-ethoxycoumarin, as shown by Rendić *et al.* [10]. Cimetidine is metabolized to a small extent [2, 3], but no K_m values have been reported. On the other hand, Sasame and Gillette [25] have demonstrated that the inhibitory effects of type II substances on the metabolism of type I substrates varies not only with the inhibitor, but also with the substrate and the animal species. As one possible reason for observing the apparent non-competitive inhibition they suggested an unusually high affinity of an inhibitor for the binding site of the enzyme, although this high-affinity binding may not be observable spectrally. However, a more likely explanation for the non-competitive inhibition suggested by Sasame and Gillette [25] was that type II substances inhibit the oxidation of type I substrates by slowing the reduction of the cytochrome P-450–substrate complex and by altering the affinity of the type I binding site for the substrate. With respect to cimetidine, the exact mechanism of its inhibitory effect remains undefined in the present study.

The prolongation of the elimination half-life of aminopyrine found here in the rat is in accordance with studies in humans, where such prolongation has been shown with warfarin, antipyrine and diazepam [8, 9, 20]. Although there is a decrease in the apparent distribution volume of aminopyrine which cannot be explained at present, and which could have some effect on the apparent half-life [26], the most probably explanation for the prolongation is the inhibition of aminopyrine metabolism by cimetidine in the liver. It remains to be shown whether the cimetidine concentrations required for this inhibition *in vitro* (between about 0.5 and 10 mM) are really attained locally in the hepatic endoplasmic reticulum. The effect of cimetidine in prolonging hexobarbital-induced sleeping time in the rat is also most probably due to the inhibition of hexobarbital metabolism in the liver.

All these findings taken together indicate that cimetidine affects the pharmacokinetics of some substances by interacting with cytochrome P-450 in the hepatic endoplasmic reticulum and thus inhibiting the metabolism of other compounds. This inhibitory

effect exhibits some specificity with respect to the species of cytochrome P-450. As to the exact mechanism of inhibition, this study and the paper by Rendić *et al.* [10] cannot give a definite answer and further studies are needed to elucidate this problem.

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