in control and treated rats, it may be suggested that BA hepatic transport was not affected by SZ. Impaired BA output in treated rats might be also a result of an alteration of the enterohepatic circulation of BA produced either by a direct effect of SZ on the intestinal cells or by the antibiotic activity of SZ on the intestinal bacteria involved in bile salt biotransformation [21]. In this connection, some antibiotics may affect the enterohepatic circulation of xenobiotics [22].

Increases in hepatic and biliary Cho in treated rats may be a consequence of decreased levels of insulin [23] induced by a diabetogenic dose of SZ, and the increase in PL biliary output (see Table 1) might be associated with that of Cho [24].

As expected [24], the infusion of TC produced increased outputs of Cho and PL in control rats though the response appeared less effective in treated animals (see Table 1).

An additional observation of interest was the decrease in the outputs of Pr and AP in SZ-treated rats and their increase during the infusion of TC in these animals (see Fig. 1). It is probably the role of BA to favor the uptake and intrahepatic transport of Pr [25] and the biliary secretion of lysosomal vesicles including enzymes like AP.* Therefore, the decrease in BA secretion produced by SZ might be involved in the decreases in Pr and AP outputs, because SZ did not affect either hepatic Pr or serum Pr (control 7.0 ± 0.2 g/100 ml, N = 4; SZ-treated, 6.7 ± 0.3 g/100 ml, N = 4). Since AP activity was increased in the livers of SZ-treated rats, it is understandable that the lysosomal enzyme accumulated may be released into bile by TC to a greater extent than Pr which, however, reached the bile levels seen in the controls.

In conclusion, SZ produced a decrease in BF at the expense of both BAIF and BADF without impairing the permeability of the biliary system. In addition, this diabetogenic compound altered the biliary secretion of lipid and protein components though the primary effect remains to be clarified. The effects induced by SZ should be considered in hepatic metabolism studies in experimental diabetes induced shortly after the administration of this compound.

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† Address correspondence to: Dr. Emilio A. Rodríguez Garay, Instituto de Fisiología Experimental, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, 2000 Rosario, Argentina.

Instituto de Fisiología Experimental
Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)
Universidad Nacional de Rosario
Rosario, Argentina

CRISTINA E. CARNOVALE
RAÚL A. MARINELLI
E. A. RODRÍGUEZ GARAY†

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Age-associated alteration in imipramine metabolism is position selective

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Imipramine is one of the widely prescribed tricyclic antidepressants. It is extensively metabolized by the hepatic microsomal cytochrome P-450 and forms 2-hydroxy imipramine and desipramine as primary metabolites by 2-hydroxylation and *N*-demethylation, respectively. In our previous study [1], we showed that the imipramine *N*-demethylase activity was about six times higher in 3-month-old male Wistar rats than in females of the corresponding age ($P < 0.01$). In contrast, there was little sex difference in imipramine 2-hydroxylase activity. These observations supported the hypothesis that these two metabolic pathways are mediated in large part by different species of

cytochrome P-450.

Recently we have demonstrated that enzyme activities such as hexobarbital hydroxylase and aminopyrine *N*-demethylase, which exhibit marked sex differences in young rats, showed a drastic decrease after 12 months in male but not in female rats, resulting in the disappearance of the sex differences in old age [2, 3]. In contrast, *p*-nitroanisole *O*-demethylase and aniline hydroxylase activities, which exhibit only small sex differences in young animals, did not show marked age-associated alterations in either male or female rats [2, 3]. Therefore, the patterns of age-associated alteration in drug metabolism are different, depending on the substrate used and the sex of the animal. Therefore, it is of interest to determine whether metabolism at different positions of a single substrate shows different age-associated alterations.

In this study, we examined the age-associated alteration in imipramine metabolism at the two positions mentioned above using male and female Fischer 344 rats.

Materials and methods

Chemicals. Imipramine hydrochloride (IMI) and desmethylimipramine hydrochloride (DMI) were kindly donated by Dainippon Pharmaceutical Co., Ltd. (Osaka); 2-hydroxyimipramine hydrochloride (2OH-IMI) was a gift from Geigy (Basel). NADP, glucose-6-phosphate (G-6-P) and G-6-P dehydrogenase were obtained commercially. All other chemicals and solvents were of analytical grade.

Animals and preparation of microsomes. SPF Fischer 344 rats of both sexes were used. They were raised in the SPF aging farm of our institute. Male and female rats with ages ranging from 3 months to 30 months were killed by decapitation and liver microsomes were prepared by ultracentrifugation (100,000 *g*, 60 min) of 9000 *g* supernatant of liver homogenates in 1.15% KCl and were washed by another 60 min ultracentrifugation [4]. Microsomal protein concentrations were determined by the method of Lowry *et al.* [5].

Assay method for IMI metabolism. The rate of oxidation of IMI at a substrate concentration of 700 μM was determined according to a previously described method [1]. A 1-ml assay mixture contained microsomes (1.0 mg protein/ml incubation mixture), 20 mM $MgCl_2$, 10 mM G-6-P, 1.2 mM NADP, 2.0 IU G-6-P dehydrogenase, 0.26 mM EDTA, and IMI in 0.15 M Tris–HCl buffer (pH 7.4). After a 5-min preincubation under air at 37°, the reaction was started by the addition of NADP. The formation of DMI and 2OH-IMI from IMI was linear up to 45 sec at the substrate concentration used in this study. The linearity of the reaction was lost after this time period due to the formation of a secondary metabolite, 2OH-DMI. Within the first 45 sec, 2OH-DMI was not detected. The incubation was therefore performed for 30 sec and stopped by the addition of 1.0 ml of 1.0 M carbonate buffer (pH 10.0). The formation of the primary metabolites of IMI in this incubation period was well within the detectable range with our high-performance liquid chromatographic (HPLC) methods.

To 1.0 ml of the stopped-reaction mixture, 125 ng of nortriptyline as internal standard and 5.0 ml of ethyl acetate were added. After extraction by vigorous mixing for 1.0 min and centrifugation for 10 min at 1200 *g*, the organic layer was transferred to another glass vial containing 500 μl of 0.1 N hydrochloric acid. The mixture was vigorously mixed for 1.0 min with a vortex mixer and centrifuged for 10 min. After discarding the organic layer, 500 μl of 1.0 M carbonate buffer (pH 10.0) and 150 μl of chloroform were added. After mixing and centrifugation as described above, 50–100 μl of the chloroform phase was injected into a Lichrosorb SI-60 column of a high-performance liquid chromatograph (model TWINCLE, Japan Spectroscopic Co., Tokyo) and the IMI metabolites, DMI, 2OH-IMI, and 2OH-DMI, were detected at 254 nm with an ultraviolet detector (model UVIDEC 100-III, Japan Spectroscopic Co.). The mobile phase consisted of methanol, acetonitrile, and ammonium hydroxide (5:35:1, by vol.), and the flow rate was 1.5 ml/min. This procedure allows simultaneous quantitative determinations of the IMI metabolites.

Results and discussion

Figure 1A shows the age-associated alteration in IMI *N*-demethylase activity in male and female Fischer 344 rats. The enzyme activities in males were about 3 times higher than those of females at ages from 3 to 12 months and then decreased up to 25 months when the activity reached the same level as that of females. In contrast, female rats maintained their 3-month activity throughout life. The IMI 2-hydroxylase activities (Fig. 1B) in male rats were slightly higher than corresponding female values up to 12 months and then gradually decreased thereafter. However, there was no notable decrease in activity in comparison to the 3-month value throughout the remainder of their lives.

In their recent clinical report, Abernethy *et al.* [6] indicated that the biological half-life of IMI was markedly prolonged in elderly subjects due to its decreased clearance, with no change in volume of distribution, while the half life of DMI was only slightly prolonged in the elderly. They speculated that the clearance of IMI, which is predominantly transformed by demethylation, may be more sensitive to the effect of age than DMI, for which the major biotransformation pathway is hydroxylation. Their speculation agrees quite well with our male rat study. A marked alteration was observed only in *N*-demethylation of IMI, and the alteration in 2-hydroxylation was very small. The age-associated alterations in DMI 2-hydroxylation should also be very small in rats, because 2-hydroxylation of DMI and IMI is mediated most likely by the same set of P-450 isozymes.*

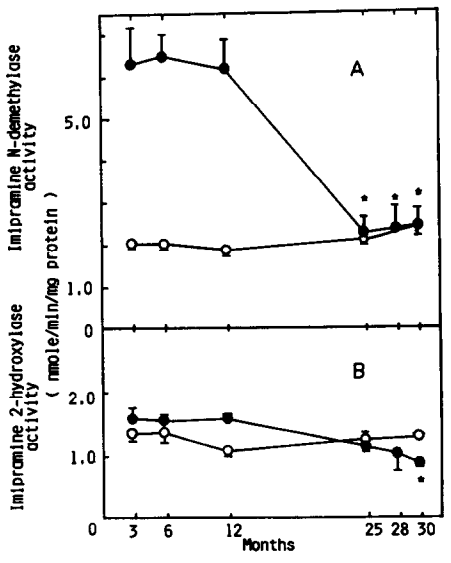

Fig. 1. Effect of age on imipramine metabolism. Age-associated alterations in *N*-demethylation (A) and 2-hydroxylation (B) of imipramine were studied using liver microsomes from male (●) and female (○) rats with ages ranging from 3 to 30 months. Data represent mean ± SE of three animals. * Significantly different from the respective 3-month-old values ($P < 0.05$).

* 2-Hydroxylation of imipramine and desipramine are mutually inhibited by competitive kinetics (M. Chiba, E. Nishihara, K. Yanai, K. Nakasa, S. Fujita, T. Suzuki and K. Kitani, 104th Annual meeting of Pharmaceutical Society of Japan, 29 March 1984, Sendai).

In summary, our present observations indicate that the patterns of the age-associated alterations in the activities of *N*-demethylation and 2-hydroxylation of IMI are quite different in male rats, and that the former showed a marked sex difference while the latter did not. Therefore, present results are consistent with the hypothesis previously proposed by ourselves that these pathways are predominantly mediated by different species of cytochrome P-450 [1].

The fact that age-associated alteration in IMI metabolism is position selective together with our previous observations on the position selective alteration of lidocaine metabolism [7], provides strong supportive evidence for the hypothesis that age-associated alterations in drug metabolizing enzyme activities are caused by alterations in the relative abundance of cytochrome P-450 species with age [2, 3]. These observations may also have important clinical and toxicological implications, because the ratio of the amounts of active metabolite, DMI and a suspected toxic metabolite 2 OH-IMI, may be different depending on age and sex.

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* *Department of Biopharmaceutics*
Faculty of Pharmaceutical Sciences
Chiba University
Chiba, Japan
† *First Laboratory of Clinical Physiology*
Tokyo Metropolitan Institute of Gerontology
35-2, Sakaecho
Itabashi-ku
Tokyo, Japan

MASATO CHIBA*
SHOICHI FUJITA*
KENICHI KITANI†
TOKUJI SUZUKI*

† Address for reprint requests: Kenichi Kitani, M.D. First Laboratory of Clinical Physiology, Tokyo Metropolitan Institute of Gerontology, 35–2, Sakaecho, Itabashi-ku, Tokyo, Japan.

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Effects of griseofulvin on enzymes associated with Phase I and II of drug metabolism

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Griseofulvin (GF), an anti-fungal agent, has been used rather effectively in man. Although this compound exhibits potent antibiotic properties, a number of side effects, particularly in liver, have been reported. In mice, GF feeding markedly alters porphyrin metabolism [1]. Enlargement and darkening of the liver and histologic evidence of porphyrinstasis and cholestasis have been reported [2, 3]. Studies by DeMatteis and Gibbs [4] reported a decrease in hepatic ferro-chelatase of mice and rats treated with GF when measured by Co^{2+}-mesoporphyrin formation in isolated mitochondria.

Studies on the effects of GF on hepatic microsomal cytochrome P-450 and cytochrome b_5 in mice and rats have been reported [5, 6]. However, more extensive studies have been done on mice [7–9]. Studies in mice by Denk *et al.* [5] showed that GF feeding results in a significant increase in hepatic cytochrome b_5 and a decrease in cytochrome P-450. Studies by Lin *et al.* [6] showed that GF treatment results in an increase in benzo[*a*]pyrene hydroxylase activity and benzphetamine demethylation in mice.

Since drugs and xenobiotics have been shown to exhibit different effects on drug-metabolizing enzymes from different animal species, studies reported in this communication were done to more fully characterize the effects of GF on both Phase I and II drug-metabolizing enzymes in rats.

Materials and methods

Animals and treatment. Male Sprague–Dawley rats weighing 120–140 g were obtained from the Holtzman Co. (Madison, WI). All animals were kept under controlled conditions (22°, lights on 6:00 a.m. to 8:00 p.m.). Animals were fed a standard powdered Purina Chow diet containing 2.5% GF for 12 days. Control animals received powdered standard diet without GF. Feeding cups were weighed daily to monitor food intake by animals in both groups. Food intake was about equal in GF and control animals.

Chemicals. NADH, NADP, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, cytochrome *c*, ferricyanide and griseofulvin were obtained from the Sigma Chemical Co. (St. Louis, MO). Benzphetamine was a gift from The Upjohn Co. (Kalamazoo, MI). All other reagents and chemicals employed were of analytical grade.

Enzyme assays. Liver microsomes were prepared following the procedure of Williams and Pendleton [10]. Total cytochrome P-450 was assayed according to the procedure of Omura and Sato [11]. Total cytochrome b_5 concentration was determined by addition of a few crystals of dithionite to the sample curvette [12]. Total heme was determined as pyridine hemochromogen according to the procedure of Paul *et al.* [13]. The metyrapone-difference spectrum of dithionite-reduced microsomes was recorded at room temperature on a Perkin–Elmer double beam, dual wavelength, scanning spectrophotometer according to the procedure of Luu-The *et al.* [14]. The assay procedures for NADPH–cytochrome *c*, NADH–cytochrome *c* and NADH–ferricyanide reductase activities of microsomes were done as previously described [15, 16]. Cytosolic glutathione *S*-transferase activity was assayed according to the procedure of Habig *et al.* [17]. Determination of benz-