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HEPATIC MICROSOMAL *N*-DEMETHYLATION OF *N*-METHYLBENZAMIDINE

N-DEALKYLATION VS *N*-OXYGENATION OF AMIDINES

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Abstract—The microsomal oxidative *N*-demethylation of *N*-methylbenzamidine, a model compound for active substances containing the basic amidine function, was investigated. *N*-Methylbenzamidine was converted into benzamidine and formaldehyde by aerobic incubation with non-induced microsomal fractions of rabbit liver homogenates and NADPH. The formation of benzamidine in the incubation mixtures under widely differing conditions was assayed using a newly-developed, high-performance, ion pair, reverse-phase partition chromatographic method. Optimal reaction conditions were determined. The benzamidine formation in the incubation mixture followed Michaelis–Menten kinetics and required the presence of molecular oxygen and NADPH. The effects of the inducer phenobarbital, methylcholanthrene, ethanol and *N*-methylbenzamidine itself on the activity were studied. Neither superoxide anion nor hydrogen peroxide was directly involved in the demethylation reaction. The direct involvement of cytochrome P-450 in this reaction is supported by the observation that the presence of inhibitors of cytochrome P-450, in particular of carbon monoxide, markedly decreased the rate of *N*-demethylation. This *N*-demethylation of *N*-methylbenzamidine proves the hypothesis that benzamidines with hydrogen atoms in the α-position to the amidine nitrogen atoms are *N*-dealkylated instead of *N*-oxygenated by the microsomal mixed function oxidase system.

Strongly basic amidine functions are components of numerous active principles [1]. In addition to the trypanocidal activity [2], the strong binding to DNA and the inhibition of tumour growth [3, 4] are worthy of mention. Furthermore, amidines are excellent inhibitors of thrombin [5]. The oxidative *N*-dealkylation of amines by microsomal enzymes represents one of the most important biotransformation processes [6]. An *N*-dealkylation of this type on strongly basic amidines has not been reported. Chlorophenamidine, a formamidine with an aromatic substituent on nitrogen and, thus, not strongly basic, has been *N*-demethylated by microsomal enzymes [7]. Amidines like *N*-methylbenzamidine possess pK_a values of 11.6 [8].

In the course of investigations on the *N*-oxygenation of benzamidines **1** to form the benzamidoximes **2** (Fig. 1A), it was found that *N*-substituted benzamidines possessing α-hydrogen atoms did not undergo *N*-oxygenation by cytochrome P-450 [8, 9]. In addition to a preference for certain tautomeric forms [9], a competing *N*-dealkylation of the *N*-alkylbenzamidines (Fig. 1B) by cytochrome P-450 was postulated as an explanation [8]. This hypothesis is confirmed by the present investigations in which we describe the *N*-demethylation of the model substance *N*-methylbenzamidine **3** by microsomal enzymes to give benzamidine **4** and formaldehyde. Using a newly-developed quantitative HPLC† method for the assay of the benzamidine **4** formed, it was possible to determine the influences of cofactors, inhibitors, inducers, and the kinetic parameters. Unambiguous evidence for the participation of cytochrome P-450 was thus found and it was also possible to compare the *N*-dealkylation with the *N*-oxygenation.

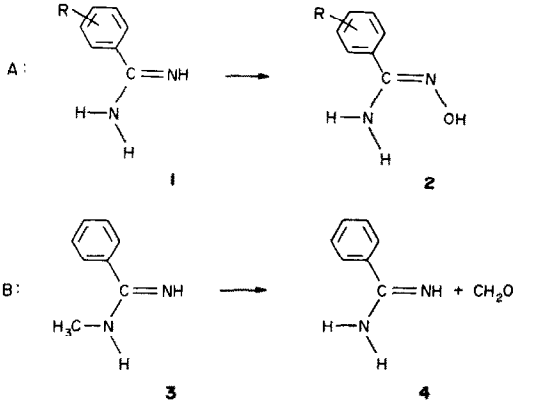

Fig. 1. *N*-Oxygenation of *N*,*N*-unsubstitued benzamidines **1** to benzamidoximes **2** (reaction A) and *N*-demethylation of *N*-methylbenzamidine **3** to benzamidine **4** (reaction B).

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† Abbreviations used: HPLC, high-performance liquid chromatography; SKF-525A, 2-diethylaminoethyl-2,2-diphenylvalerate hydrochloride.

MATERIALS AND METHODS

Reagents and biochemicals. NADPH (tetrasodium salt), NADP (disodium salt), glucose-6-phosphate dehydrogenase suspension (purity grade 1), and catalase were purchased from Boehringer-Mannheim GmbH (Mannheim, F.R.G.). Superoxide dismutase from bovine erythrocytes (3000 U/mg) was obtained from Serva (Heidelberg, F.R.G.). 3-Methylcholanthrene was a product from Sigma (Taufkirchen, F.R.G.). SKF 525-A† was kindly supplied by Professor K. Netter (Marburg, F.R.G.). 1-Octane-sulfonic acid, sodium salt, benzamidine hydrochloride, and metyrapone were purchased from EGA-Chemie (Steinheim, F.R.G.). All other chemicals and solvents (GR) were obtained from Merck AG (Darmstadt, F.R.G.).

Synthesis. *N*-Methylbenzamidine was prepared by the method of Pinner and isolated as the hydrochloride [10].

Preparation of liver homogenates. Untreated rabbits of either sex [1.8–2.9 kg) were used and fed on a standard diet ("Altromin", supplied by Lage, F.R.G.). They were allowed drinking water *ad libitum*. Rabbits were stunned, decapitated, and the livers removed immediately. All subsequent operations were carried out at 0–4°. The livers were washed three times with 0.9% (w/v) saline solution, blotted dry, weighed, and minced with scissors. The minced livers were homogenized with 4 vol. of 1.15% (w/v) KCl solution (0.154 M) using a motorized Teflon pestle glass tube homogenizer (Potter S, for 30 ml, B. Braun Melsungen AG) or alternatively with an Ultra-Turrax homogenizer (3 × 15 sec, interrupted by cooling in an ice bath for 1 min). Both procedures gave tissue fractions with similar specific activities. The homogenates were transferred to plastic tubes and centrifuged at 12,000 *g* for 25 min. The supernatant was carefully decanted and used as the enzyme source. The preparations were stored at −20° and could be used for three weeks without a significant loss of activity. Microsomes and the 100,000 *g* supernatant were prepared immediately before use by the procedure of Omura and Sato [11]. Protein concentrations were measured using the method of Gornall *et al.* [12]. The content of cytochrome P-450 in the microsomes was determined as described by Omura and Sato [13]. The formation of formaldehyde in the incubation mixture was confirmed by the method of Nash [14].

Xenobiotic treatment. Rabbits of either sex [1.8–2.9 kg) were used. Animals were injected i.v. with a saline solution of 60 mg phenobarbital sodium salt/kg body weight once daily for five consecutive days. Control animals received only 0.9% saline solution. *N*-Methylbenzamidine hydrochloride dissolved in 0.9% (w/v) saline was given i.v. twice a day for five consecutive days at doses of 30 mg/kg body weight. Control animals received only saline solution. Animals were injected intraperitoneally with a corn oil solution of 25 mg 3-methylcholanthrene/kg body weight once daily for two days and twice on the third day. Control animals received only corn oil. Rabbits were given drinking water containing 10% ethanol (v/v) for 1 week followed by 5% ethanol (v/v) for an additional 2 weeks (the total amount of ethanol uptake were, respectively, 160 and 195 mg). In all protocols the animals were fasted for 24 hr before they were killed. The microsomal fractions were prepared as described above.

Incubations. Incubations were carried out in unstoppered 25 ml Erlenmeyer flasks at 37° in a shaking water-bath. The standard incubation mixture (total vol. 5.5 ml) contained the following components: 3.0 ml of phosphate buffer (pH 7.4; 8.7 mM KH_2PO_4, 30.4 mM Na_2HPO_4), 12,000 *g* supernatant corresponding to 10 mg of protein, 1.0 ml of usually 20 mM *N*-methylbenzamidine hydrochloride solution in phosphate buffer (pH 7.4), and 1.0 ml of cofactor solution (containing NADPH, 2 mg; $MgCl_2$, 1.9 mg; and phosphate buffer pH 7.4 to a final vol. of 1.0 ml). Since preliminary experiments had shown that incubation of microsomes either in the presence of NADPH or of an NADPH-generating system gave similar results, NADPH was used in all of the experiments reported. Two mg of NADPH was found to be the optimum amount for the reactions at the selected concentrations as a doubling of the amount of NADPH did not result in an increase in the reaction rate. After preincubation at 37°, the reaction was started by addition of the 12,000 *g* supernatant. A 30 min incubation time was routinely employed. Incubations were terminated by mixing thoroughly with 4.0 ml of freshly-distilled acetonitrile and the protein was separated by centrifugation at 3000 *g* for 10 min.

High-performance liquid chromatography. Aliquots (2–5 μl) of the terminated incubations were directly analysed using a high pressure liquid chromatograph with an automatic injection system WISP 170 A (Waters Associates, Eschborn, F.R.G.) and equipped with a variable wave-length detector set at 229 nm (UV-detector ERC-7210, Erma Optical Works, Ltd, Alteglofftheim, F.R.G.). Separation and quantification of benzamidine were performed at room temperature on a prepacked RP-8 column (250 mm length × 4.6 mm i.d., particle size 7 μm, Dr. Knauer KG, Oberursel, F.R.G.). A protecting-column RP-8 (Dr. Knauer KG, Oberursel, F.R.G.), placed immediately before the analytical column, was used routinely. The mobile phase was acetonitrile/water/phosphoric acid 85%/1-octanesulfonic acid, sodium salt (60:40:0.08:0.0018 M, v/v/v/M). This mobile phase flowed through the column at the rate of 1.0 ml. Solvents used in the analyses were filtered through a membranous filter (RC-255, Schleicher and Schuell, F.R.G.) and degassed under vacuum. Standard curves (peak height) at the level of 10, 20, 40, 60, 80, 100, 160, and 200 nmol benzamidine were constructed by introducing known amounts of benzamidine into the usual incubation mixtures (20 μmol *N*-methylbenzamidine, omission of NADPH), the mixtures were then incubated and treated in the same way as the experimental samples. Standard curves were linear over this range with correlation coefficients of 0.995 or greater. The levels of benzamidine in unknown incubation mixtures were determined directly from these standard curves which were run in parallel with the experimental samples. The recovery of benzamidine from incubation mixtures fortified with benzamidine was 96.5 ± 4.2% (N = 32) of that obtained using samples

which contained the same amount of benzamidine dissolved in acetonitrile/water (60:40, v/v). The detection limit of benzamidine in one incubation mixture is 10 nmol which corresponds to a rate of N-demethylation of 0.033 nmol benzamidine. HCl/min per mg protein (incubation time 30 min, 20 μmol substrate, 10 mg protein).

Carbon monoxide inhibition. Three flowmeters (manometers, Rota, Wehr, F.R.G.) with adjustable hair-pin valves are linked to the particular gas cylinders (O_2, N_2, CO). The outlets of the manometers were combined and terminated in a gas line which was the connection to the reaction vessel. One ml of 12,000 *g* supernatant, 2.0 ml of phosphate buffer (pH 7.4), and 1.0 ml of 20 mM N-methylbenzamidine hydrochloride solution were mixed in an ice-cooled, three-necked flask fitted with a gas distribution tube, a dropping funnel with pressure relief, and a gas outlet (sintered glass plug filled with paraffin). The dropping funnel contained 2 ml of a degassed cofactor solution (2 mg NADPH and 1.9 mg $MgCl_2$ in phosphate buffer pH 7.4). The mixture in the flask was purged with nitrogen for 2 min and then with the particular gas mixture of O_2, N_2, and CO. The reaction was started by the addition of the cofactor solution and heating to 37°. During the incubation (30 min), the incubation mixture was stirred and the flask was gassed with the CO, O_2, N_2-mixture. Incubations were terminated by adding 4.0 ml of freshly-distilled acetonitrile and were analysed by HPLC.

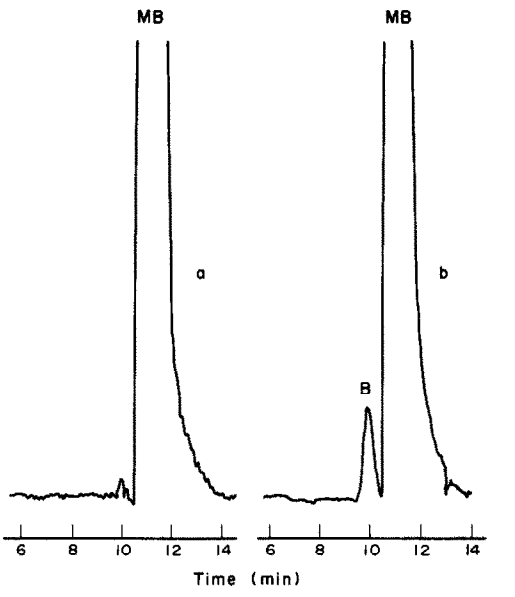

Fig. 2. Representative HPLC chromatogram of N-methylbenzamidine metabolism by 12,000 *g* supernatant fractions of rabbit liver homogenates. See Materials and Methods for details of reaction mixture content, incubation, sampling and analysis. Key: (a) omission of NADPH; (b) complete system; MB, N-methylbenzamidine; and B, benzamidine.

RESULTS

Quantitative analysis of benzamidine formed by microsomal N-demethylation of N-methylbenzamidine

The identity of the formaldehyde formed by the microsomal incubation of N-methylbenzamidine was established by the reaction reported by Nash [14]. Because of the low sensitivity of this assay [15], this method was not suitable for the quantitative characterisation of reaction B (Fig. 1B). The analysis of benzamidine in the microsomal incubation mixtures was complicated by the large excess of N-methylbenzamidine. The chromatographic system must be able to allow a separation between methylbenzamidine and benzamidine in the range of 100:1. This was made possible by a newly developed HPLC-analysis (Fig. 2). The addition of 1-octane-sulfonic acid to the mobile phase proved to be successful and thus the separation was performed by the ion-paired chromatography technique.

Microsomal enzymes, cofactor requirement, and effect of inhibitors

Of all the fractions of rabbit liver homogenates tested, only the 12,000 *g* supernatant and the microsomes showed a measurable activity (Table 1). It was also found that the reaction was proportional to microsomal protein concentration in the incubation mixtures (Table 2). The reactions required the presence of NADPH in addition to oxygen. Addition of magnesium chloride resulted in a higher activity. The inhibitors of the cytochrome P-450 system [16] SKF 525A, potassium cyanide, metyrapone (Table 1), and carbon monoxide (Table 3) inhibited the microsomal N-demethylation of N-methylbenzamidine.

The influence of carbon monoxide was more thoroughly studied. The proportions of CO to O_2 were varied and the respective inhibition in comparison to a control determined. It was ensured that the respective oxygen concentration (also in the control experiment) remained constant. Thus, it can be

Table 1. Effect of cofactors and of inhibitors of cytochrome P-450 on the N-demethylation of N-methylbenzamidine by rabbit liver microsomal enzymes

Enzyme	Incubation mixture	nmol benzamidine HCl/min per mg protein
12,000 *g* supernatant	Complete	0.45 ± 0.17
	−NADPH	<0.033
	$-Mg^{2+}$	0.31 ± 0.13
	$-O_2$	0.12 ± 0.07
	+SKF 525 (5 mM)	<0.033
	+KCN (25 μM)	0.38 ± 0.14
	+Metyrapone (0.1 mM)	0.16 ± 0.06
	25°	0.13 ± 0.03
Microsomes 100,000 *g* supernatant	Complete*	0.40 ± 0.11†
	Complete	<0.033

Values are presented as the means of determinations from three animals ± SD; reaction mixtures contained the components described in Materials and Methods, except for the omission of cofactors or the addition of inhibitors; *microsomes equivalent to 10 mg protein; †0.27 ± 0.07 nmol benzamidine·HCl/min per nmol cytochrome P-450.

Table 2. Effects of various amounts of microsomal protein on the liver microsomal enzymatic *N*-demethylation of *N*-methylbenzamidine to benzamidine

Microsomal protein (mg)	nmol benzamidine·HCl/min
0	0
2.3	1.18 ± 0.43
4.6	2.18 ± 0.76
6.9	3.25 ± 1.01
9.2	4.33 ± 1.44

Values are presented as the means of determination from three animals ± SD; incubations were carried out as described under Materials and Methods.

excluded that a supposed inhibition is only a result of a decreased amount of available oxygen [17]. The data given in Table 3 confirm the inhibition by carbon monoxide.

Kinetics of the N-*demethylation of* N-*methylbenzamidine*

The formation of benzamidine was linear over a period of 30 min and, thus, an incubation time of 20–30 min was chosen for the various studies (Fig. 3). The *N*-demethylation of *N*-methylbenzamidine during the incubation with the 12,000 *g* supernatant of rabbit liver homogenates and NADPH followed Michaelis–Menten kinetics. The apparent K_m value, calculated from the Lineweaver–Burk plot (Fig. 4), was 19.8 ± 1.0 mM, while the apparent V_{max} value was found to be 1.71 ± 0.07 nmol benzamidine produced/min per mg of protein (values obtained with liver preparations from two other rabbits were, respectively, 23.4 ± 0.4 mM, 28.2 ± 0.5 mM, and 2.16 ± 0.10, 2.05 ± 0.12 nmol benzamidine formed/min per mg of protein; the average values ± SD of the three different experiments (three different animals) were, respectively, 23.8 ± 4.2 mM and 1.98 ± 0.23 benzamidine formed/min per mg of protein).

Table 3. Effect of various CO/O_2-concentrations on the liver microsomal enzymatic *N*-demethylation of *N*-methylbenzamidine to benzamidine

CO/O_2	%CO	$\%O_2$	$\%N_2$	% of control activity
Control	0	5	95	100
1	5	5	90	78
2	10	5	85	60
3	15	5	80	48
5	25	5	70	27
8	40	5	55	19

Values are presented as the means of three determinations (three separate samples of one homogenate); liver preparations from two other animals gave similar results. Incubations were carried out as described under Materials and Methods.

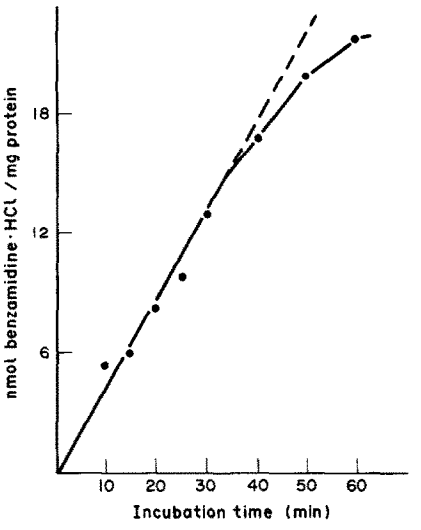

Fig. 3. Formation of benzamidine by 12,000 *g* supernatant fractions of rabbit liver homogenates as a function of time. Each point is the mean of three incubations. See Materials and Methods for details of reaction mixture content, incubation, sampling, and analysis.

Effects of induction on N-*demethylation*

Pretreatment of rabbits with phenobarbital did not significantly change the rate of *N*-demethylation of *N*-methylbenzamidine. Only a slight increase in the specific rate of reaction was detected (Table 4).

It was also examined whether or not benzamidine is able to induce its own biotransformation. After *in vivo* pretreatment with *N*-methylbenzamidine, no significant changes of the microsomal *N*-demethylation activity could be detected (Table 5). So far no final explanation of the seeming decrease in

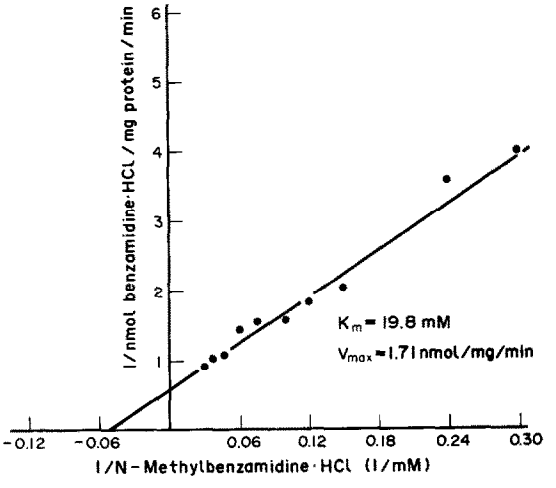

Fig. 4. Lineweaver–Burk plot of *N*-demethylation of *N*-methylbenzamidine measured by benzamidine formation in the reaction mixture. Each point is the mean of three determinations. The mixtures contained the components described in Materials and Methods.

Table 4. Effect of administration of phenobarbital on the *N*-demethylation of *N*-methylbenzamidine by rabbit liver microsomes and on the content of cytochrome P-450

Treatment	nmol cytochrome P-450/mg protein	nmol benzamidine·HCl/min per nmol cytochrome P-450	nmol benzamidine·HCl/min per mg protein
Control	1.61 ± 0.13	0.28 ± 0.04	0.47 ± 0.07
Phenobarbital	5.02 ± 0.10	0.19 ± 0.07	0.85 ± 0.28

Values are presented as the means of determinations from three animals ± SD; xenobiotic treatment and incubations were carried out as described under Materials and Methods.

Table 5. Effect of pretreatment with benzamidines on the *N*-demethylation of *N*-methylbenzamidine by rabbit liver microsomes and on the content of cytochrome P-450

Treatment	nmol cytochrome P-450/ mg protein	nmol benzamidine·HCl/min per nmol cytochrome P-450	nmol benzamidine·HCl/min per mg protein
Control	2.08 ± 0.35	0.23 ± 0.08	0.46 ± 0.10
N-methylbenzamidine	1.53 ± 0.33	0.29 ± 0.04	0.43 ± 0.03

Values are presented as the means of determinations from two animals ± SD; xenobiotic treatment and incubations were carried out as described under Materials and Methods.

Table 6. Effect of chronic ingestion of ethanol on the *N*-demethylation of *N*-methylbenzamidine by rabbit liver microsomes and on the content of cytochrome P-450

Treatment	nmol cytochrome P-450/ mg protein	nmol benzamidine·HCl/min per nmol cytochrome P-450	nmol benzamidine·HCl/min per mg protein
Control	1.41 ± 0.28	0.36 ± 0.03	0.51 ± 0.02
Ethanol	1.33 ± 0.11	0.40 ± 0.04	0.53 ± 0.07

Values are presented as the means of determinations from two animals ± SD; xenbiotic treatment and incubations were carried out as described under Materials and Methods.

Table 7. Effect of administration of 3-methylcholanthrene (3-MC) on the *N*-demethylation of *N*-methylbenzamidine by rabbit liver microsomes and on the content of cytochrome P-450

Treatment	nmol cytochrome P-450/ mg protein	nmol benzamidine·HCl/min per nmol cytochrome P-450	nmol benzamidine·HCl/min per mg protein
Control	1.24 ± 0.01	0.40 ± 0.06	0.49 ± 0.07
3-MCl	2.68 ± 0.14	0.08 ± 0.01	0.21 ± 0.04

Values are presented as the means of determinations from two animals ± SD; xenobiotic treatment and incubations were carried out as described under Materials and Methods.

Table 8. Effects of addition of superoxide dismutase, catalase and H_2O_2 on the rate of *N*-methylbenzamidine *N*-demethylation by 12,000 *g* supernatant fractions of rabbit liver homogenates

Addition	nmol benzamidine·HCl/min per mg protein	% of control activity
None	0.45 ± 0.17	100
Superoxide dismutase*	0.43 ± 0.18	96
Catalase*	0.45 ± 0.17	100
H_2O_2†	<0.033	0

Values are presented as the means of determinations from three animals ± SD; *superoxide dismutase (2.0 mg) or catalase (2.0 mg) was added to the reaction mixture containing the components described in Materials and Methods; †0.1 ml 30% H_2O_2 was added to the reaction mixture containing the components described in Materials and Methods but without NADPH.

cytochrome P-450 content in microsomes from rabbits treated with *N*-methylbenzamidine can be given. It might be a depression of this synthesis *in vivo*.

Chronic ethanol treatment of rabbits did not increase the metabolism of *N*-methylbenzamidine. No change in the specific rate of reaction could be observed (Table 6). Specific isoenzymes which are inducible by compounds like 3-methylcholanthrene do not play a part in the *N*-demethylation of *N*-methylbenzamidine as the specific rate of reaction was significantly lower after pretreatment of rabbits with this compound (Table 7).

Influence of catalase and superoxide dismutase

Table 8 shows the effects of the addition of superoxide dismutase, catalase, and hydrogen peroxide on the rate of *N*-demethylation. Taking the experimental errors into consideration, the effects of these agents seemed to be not significant. Therefore, superoxide and hydrogen peroxide may not be involved directly in the *N*-demethylation.

DISCUSSION

It was possible with the help of a newly-developed HPLC analysis methods to prove and to characterise the *N*-demethylation of the model compound *N*-methylbenzamidine. On the basis of the data thus obtained it is clear that the *N*-demethylation of *N*-methylbenzamidine exhibits the typical properties of a microsomal monooxygenase system, which requires NADPH and O_2 in addition to microsomal proteins and which shows increased activity in the presence of Mg^{2+} ions.

There is almost universal agreement that the oxidative *N*-dealkylation of aliphatic amines is catalysed by specific isoenzymes of the cytochrome P-450 family of monooxygenases whereas *N*-oxygenation can also be performed by the flavin-containing monooxygenase [18]. Amidines are not substrates for purified microsomal FAD-containing monooxygenase [19]. In the present study on the basis of the

inhibition of the *N*-demethylation of *N*-methylbenzamidine by typical inhibitors of the cytochrome P-450 monooxygenase system (Table 1) and especially by carbon monoxide (Table 3), evidence is obtained that cytochrome P-450 is also involved in the *N*-dealkylation of strongly basic amidines. The responsible isoenzyme of the cytochrome P-450 system is obviously a constitutive form which is not further inducible as studies with phenobarbital (Table 4), *N*-methylbenzamidine itself (Table 5), ethanol (Table 6) and 3-methylcholanthrene (Table 7) demonstrated, that only after pretreatment of rabbits with phenobarbital a slight but not very significant increase in the specific rate of reaction could be observed. The *N*-demethylation of amidines is obviously catalysed by different isoenzymes than the *N*-demethylation of methylguanidine, another strongly basic substrate, as the rate of guanidine and formaldehyde formation was significantly higher with the microsomes from phenobarbital-treated rabbits than with those from untreated rabbits [20].

In addition, an H_2O_2-supported enzymatic *N*-dealkylation (peroxidatic function of liver microsomal cytochrome P-450), as has been described for other oxidative *N*-dealkylation reactions [21], can be disregarded (Table 8). Just as H_2O_2 alone is not able to convert *N*-methylbenzamidine to benzamidine, the NADPH in the incubation experiments also cannot be replaced by H_2O_2. Additions of catalase or superoxide dismutase have no significant influence on the reactions, thus supporting the present findings.

The V_{max} values for the *N*-demethylation of *N*-methylbenzamidine can still be correlated with other enzyme activities of the cytochrome P-450 monooxygenase system since most organic drug substrates have values between 1.0 and 15.0 nmol product formed per minute per milligram of microsomal protein [15]. The apparent K_m value of 19.8 mM is relatively high but still comparable with data found for other foreign substrates of the unspecific cytochrome P-450 monooxygenase system [22, 23].

It must be mentioned, however, that the rates of *N*-demethylation of aliphatic amines [24] are certainly higher than those of the studied amidines. One reason for this could be the high basicity and the poor fat solubility of the amidines [24]. One objective of the present investigation was to contribute to the solution of the problem of the predictability of *N*-oxidative biotransformation processes. Taking into consideration the widely different reports in this field, the hypothesis can be made that, especially for the *N*-oxygenation of amines, reactions of this type are particularly catalysed by the cytochrome P-450 enzyme system when an *N*-dealkylation (absence of H atoms on the α-carbon atoms) is not possible; radical mechanisms have been suggested as an explanation for this [8, 18, and references cited therein]. This concept was also derived on the basis of studies on a large number of differently substituted benzamidines [8] as *N,N*-unsubstituted benzamidines and *N*-phenylbenzamidine (absence of α–H atoms) were *N*-oxgenated whereas *N*-alkylbenzamidines such as *N*-methylbenzamidine were not [8]. Evidence that *N*-methylbenzamidine underwent, instead, an *N*-dealkylation (Fig. 1B) was obtained in this study. Furthermore, the characteristics for the reaction B found here (Fig. 1B) can be compared very well with those of the *N*-oxygenation of benzamidine (Fig. 1A) which were determined in an analogous investigation [25]. Both microsomal-, NADPH- and O_2-dependent reactions are not inducible by phenobarbital, are not influenced by superoxide dismutase, catalase, or H_2O_2, react similarly towards inhibitors, especially CO, and exhibit comparable kinetic parameters, whereby slight differences can be accounted for by differing affinities of the two substrates **1** and **3** (additional methyl group) to the enzyme. Apparently both reactions are catalysed by the same isoenzyme of the cytochrome P-450 system.

Now two good model reactions A and B (Fig. 1) are available which allow the two processes to be recognised as competing reactions. Correlations with other *N*-oxygenations are more difficult as, in contrast to the case of benzamidoxime **2**, the *N*-oxygenated product primarily formed by the action of cytochrome P-450 can very often undergo further chemical or enzymatic transformations [26, 27].

The competing reactions *N*-dealkylation and *N*-oxygenation—both catalysed by isoenzymes of the cytochrome P-450 system—are being investigated further at present on the example of benzamide. Thus, as predicted, *N*-methylbenzamidine competitively inhibits the *N*-oxygenation of benzamidine‡ and the substrate *N*-tert.-butylbenzamidine (Absence of α–H atoms) is *N*-oxygenated [28].

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‡ Publication in preparation.

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