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INHIBITION OF CYTOCHROME P-450-DEPENDENT OXIDATION REACTIONS BY MAO INHIBITORS IN RAT LIVER MICROSOMES

HERVÉ DUPONT*†, DONALD S. DAVIES* and MARGHERITA STROLIN-BENEDETTI‡

* Department of Clinical Pharmacology, Royal postgraduate Medical School, London W12 0HS, U.K., and ‡ Laboratoires Delalande, Centre de Recherches, 10 rue des Carrières, 92500 Rueil-Malmaison, France

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Abstract—The inhibition of cytochrome P-450 dependent hydroxylations of bufuralol (BH) and antipyrine, and *O*-deethylation of 7-ethoxycoumarin (7-ECOD) by several monoamine oxidase inhibitors (MAOIs) was investigated in rat liver microsomes. According to their IC_{50} values, clorgyline was the most potent inhibitor while toloxatone, the only reversible MAOI in this study, was the least potent. A great variability of inhibitory potencies was found, even in the same chemical class of MAOIs. Irreversible inhibition of BH and 7-ECOD has been studied. Rapid irreversible inhibition occurred in some cases, and this could be responsible for *in vivo* inhibition after repeated dosing of these MAOIs.

Interactions have been observed between monoamine oxidase (MAO§) inhibitors and concomitantly administered drugs [1]. In some cases, the inhibition of liver drug metabolizing enzymes by MAOIs has been proposed [2, 3]. Since metabolism of drugs by cytochrome P-450 enzymes often facilitates their elimination or makes the parent compound pharmacologically inactive, inhibition of the normal metabolic pathway may increase the intensity and duration of action of drugs.

Data from *in vitro* experiments [4–8] have shown inhibition of cytochrome P-450-dependent reactions by some MAO inhibitors, but their potencies are greatly variable. The present study was designed to compare a number of old and new MAO inhibitors with respect to their abilities to inhibit the hydroxylation of bufuralol (BUF) and antipyrine (AP) as well as *O*-deethylation of 7-ethoxycoumarin (7-EC) by rat liver microsomes. The reversibility of these inhibitory effects was also investigated.

MATERIALS AND METHODS

Chemicals. Phenelzine sulfate, pargyline hydrochloride, iproniazid phosphate, tranylcypromine hydrochloride, NADP, NADPH, D-glucose-6-phosphate monosodium salt, glucose-6-phosphate dehydrogenase and bovine serum albumin (fraction V) were all from Sigma Chemical Co. (Poole, U.K.). Clorgyline hydrochloride, l-deprenyl hydrochloride and toloxatone‖ were provided by Delalande Research Centre (Rueil-Malmaison, France). Isomers (+) and (−) of tranylcypromine were a generous gift from Röhm-Pharma. 7-Ethoxycoumarin (Gold Label grade), 7-hydroxycoumarin and 4-hydroxy-antipyrine were from Aldrich Chemical Co. Ltd (Gillingham, U.K.). Bufuralol hydrochloride and the oxalate of 1′-hydroxybufuralol were generous gifts of Roche Products plc (Welwyn Garden City, U.K.). [*N*-methyl-^{14}C]Antipyrine was from Amersham International plc (Amersham, U.K.) and 3-hydroxy-methyl-antipyrine was generously provided by Professor Breimer (University of Leidin, The Netherlands). All solvents were of analytical reagent grade except acetonitrile and methyl-tert-butyl-ether (MTBE), which were of HPLC grade, and 2-methoxy-ethanol which was of Glass Distilled grade.

Preparation of microsomes. Male Wistar rats (Olac Ltd, U.K.) weighing about 200 g were used. Microsomes were prepared as previously described [9]. Four livers were pooled for each preparation. The homogenate, in 10 vol. of 0.25 mol/l potassium-phosphate buffer, pH 7.25, containing 0.15 mol/l KCl and 1 mmol/l EDTA, was centrifuged at 10,000 *g* for 15 min. The supernatant was centrifuged at 120,000 *g* for 1 hr, then the pellet was resuspended in 0.25 mol/l potassium-phosphate buffer, pH 7.25, containing 30% (v/v) glycerol and aliquots were frozen at −80° until required.

The concentration of microsomal proteins was determined by the method of Lowry *et al.* [10], with crystalline bovine serum albumine fraction V as standard.

Measurements of enzyme activities. All reactions were started by addition of microsomes and performed at 37° in a shaking water bath. All inhibitors were added as aqueous solutions.

Bufuralol 1′-hydroxylase activity was measured as described by Boobis *et al.* [11] with minor modi-

† All correspondence should be addressed to Hervé Dupont, Laboratoires Fournier, Centre de Recherches, 50 rue de Dijon, Daix, 21121 Fontaine-lès-Dijon, France.

§ Abbreviations used: MAO, monoamine oxidase, MAOI, monoamine oxidase inhibitor; AP, antipyrine; BUF, bufuralol; 7-EC, 7-ethoxycoumarin; BH, bufuralol hydroxylase; 7-ECOD, 7-ethoxycoumarin *O*-deethylase; MTBE, methyl tert-butyl ether.

‖ Humoryl®, Delalande Laboratories, France.

fications. The incubation mixture, in a total volume of 0.25 ml, comprised 50 mmol/l Tris–HCl buffer, pH 7.4, 5 mmol/l $MgCl_2$, 2.4 mmol/l NADPH and 0.02 mg/ml of microsomal proteins. Linearity of product formation with time was checked. Bufuralol was added at concentrations ranging from 3 to 250 μmol/l for the determination of the kinetic parameters of the hydroxylation reaction. 10 μmol/l bufuralol was then used for the inhibition experiments. After 2 min incubation, the reaction was terminated by addition of 2 ml of ice-cold methyl tert-butyl ether (MTBE) which was also the extraction reagent. Ten microlitres of 5 N NaOH were added, then the samples were vortex mixed for 15 sec. The phases were separated by centrifugation and the organic phase transferred for HPLC analysis.

1′-Hydroxybufuralol was measured by HPLC-fluorescence spectrometry. The column was a straight phase Spherisorb 5 μm silica, 25 cm × 4 mm (Shandon Southern Products Ltd, U.K.). The mobile phase was 80% MTBE, 20% acetonitrile containing 0.04% perchloric acid. This was delivered at a flow rate of 2 ml/min by a Waters pump. The injection volume, via a Rheodyne 100 p injector (Anachem Ltd, U.K.), was 250 μl. Detection of 1′-hydroxybufuralol was by fluorescence spectrometry (Model LS4,Perkin-Elmer Ltd, U.K.) with an excitation wavelength of 244 nm and an emission wavelength of 312 nm. Both excitation and emission slits were set to 10 nm. Quantitation was from peak height, recorded on a Tekman dual pen potentiometric recorder (Anachem Ltd, U.K.), using a calibration curve with known concentrations of 1′-hydroxybufuralol between 0.1 and 1.5 μmol/l.

7-Ethoxycoumarin *O*-deethylase (7-ECOD) activity was measured by the method of Greenlee and Poland [12] with minor modifications. The incubation mixture, in a total volume of 1 ml, comprised 64 mmol/l potassium phosphate buffer, pH 7.2, 0.5 mmol/l NADPH, 5 mmol/l $MgCl_2$ and 0.1 mg/ml of microsomal proteins. Linearity of product formation with time was checked. 7-EC was added in 10 μl of methanol at final concentrations ranging from 0.5 to 1000 μmol/l for determining kinetic parameters of the enzyme reaction. Two and 500 μmol/l 7-EC were used for inhibition experiments. After 5 min incubation, the reaction was stopped by addition of 5 ml of ice-cold chloroform and vigorous shaking for 20 sec. Separation of phases was performed by centrifugation. The organic phase was back extracted in 1 ml of 0.01 N NaOH containing 1 mol/l NaCl. Storage in this solution did not exceed 20 min because of the instability of hydroxycoumarin. Hydroxycoumarin was quantified fluorometrically using an Aminco-Bowman spectrophotofluorimeter set at 368 nm for excitation and 456 nm for emission. A calibration curve was established with known concentrations of the metabolite ranging from 5 to 800 nM.

Antipyrine (AP) hydroxylation to 4-hydroxy-antipyrine and 3-hydroxy-methylantipyrine were assessed by a radiometric method. One microCurie of [^{14}C]-AP in 20 μl methanol was taken and the solvent was blown off under a stream of nitrogen. Unlabelled AP was then added in buffer in order to give a total concentration of 2 mmol/l. The incubation mixture, in a total volume of 250 μl, comprised 1.2 mmol/l NADPH, 6 mmol/l $MgCl_2$ and 1 mg/ml of microsomal proteins in 50 mmol/l Tris–HCl buffer pH 7.4. Linearity of product formation with time was checked. The reaction was terminated after 7 min of incubation by addition of 250 μl of 1 mol/l NaOH containing 40 mg/ml of the antioxidant $Na_2S_2O_5$. Unlabelled metabolites (20 μg of each) were added in 20 μl methanol as recovery standards. AP was removed by 3 repeated extractions with 5 ml of toluene. The pH of the aqueous phase was then adjusted to 7.0 by addition of 1 ml of 1 mol/l potassium phosphate buffer, pH 7.0. The metabolites, 4-hydroxy-antipyrine and 3-hydroxy-methyl-antipyrine, were extracted into 5 ml of chloroform/ethanol 9:1 (v/v) according to Teunissen *et al.* [13]. The organic phase was collected and evaporated to dryness under a stream of nitrogen. The residue was dissolved in 250 μl of mobile phase and 200 μl were injected for HPLC analysis.

The HPLC column was a reverse phase Spherisorb 5 μm C8 (Browlee) 25 cm × 4.6 mm and the mobile phase was 15% acetonitrile, 12% 2-methoxyethanol in 0.02 mol/l potassium phosphate buffer at a final pH of 7.55 to 7.7. The flow rate was 1.5 ml/min. Fractions corresponding to the metabolites were detected by a Hitachi u.v. detector set at 254 nm and collected for counting radioactivity. Calibration curves were constructed with concentrations of metabolites ranging from 0.02 to 0.08 g/l.

Preincubation experiments. Microsomes (1 mg protein/ml) were preincubated with or without a MAOI, in presence or in absence of an NADPH-regenerating system which comprised 0.5 mmol/l NADP, 2 units/ml of glucose-6-phosphate dehydrogenase and 8 mmol/l of glucose-6-phosphate. In either case, the preincubation mixture contained 1 mmol/l EDTA and 6 mmol/l $MgCl_2$ in 0.1 mol/l potassium phosphate buffer, pH 7.4. Preincubations were started by addition of microsomes and were performed in 50 ml tubes in a maximum volume of 5 ml at 37° in a shaking water bath at 60 cycles/min. Aliquots were withdrawn at various times of preincubation, diluted 1:5 in ice-cold 0.28 mol/l potassium phosphate buffer (0.25 mol/l final), pH 7.25 containing 1 mmol/l EDTA, and centrifuged at 120,000 *g* for 50 min. The pellet obtained was resuspended in the appropriate buffer for measuring cytochrome P-450-dependent activities remaining.

Determination of kinetic parameters. K_m and V_{max} of BH and 7-ECOD were calculated by an iterative program based on a non-linear least squares regression analysis [14] to fit the Michaelis–Menten equation for a biphasic enzyme system.

Apparent pseudo-first order rate constants (k'_{app}) of irreversible inhibitions are the slope of the graphs plotting ln (% inhibition) versus time.

RESULTS

Characteristics of the activities tested

Bufuralol 1′-hydroxylase and 7-ethoxycoumarin *O*-deethylase activities were measured with a range of substrate concentrations of 3–250 μmol/l and 0.5–1000 μmol/l respectively. When the velocity of the reaction was plotted against substrate concentra-

Table 1. Kinetic parameters of microsomal reactions

Reactions	Kinetic parameters	
Antipyrine 4-hydroxylation*	K_m = 4.59 mmol/l V = 1.71 nmol/mg/min	
Antipyrine 3-hydroxylation*	K_m = 2.22 mmol/l V = 3.80 nmol/mg/min	
7-Ethoxycoumarin *O*-deethylation	K_{m_1} = 1.4 μmol/l V_1 = 49 pmol/mg/min	K_{m_2} = 420 μmol/l V_2 = 980 pmol/mg/min
Bufuralol 1'-hydroxylation	K_{m_1} = 1.01 μmol/l V_1 = 1.19 nmol/mg/min	K_{m_2} = 157 μmol/l V_2 = 2.72 nmol/mg/min

* These values are from ref. 9.

Table 2. IC_{50} (μmol/l) of inhibitors towards microsomal reactions

	Reactions					
	Antipyrine		7-Ethoxycoumarin *O*-deethylation			
Inhibitors	3-hydroxyl.	4-hydroxyl.	Low K_m activity	High K_m activity	Bufuralol 1'-hydroyl.	R1*
Toloxatone	N.D.	N.D.	2000	6000	12000	6
Iproniazid	250	350	2500	1900	1400	10
Pargyline	250	250	950	750	3200	12.8
(−)Deprenyl	52	60	900	450	N.D.	17.3
Tranylcypromine	20	25	18	65	130	7.2
Phenelzine	18	24	120	4.5	190	42.2
Clorgyline	5.8	6.5	2.1	45	13	21.4
R_2*	43.1	53.8	1190	1333	923	

* R1 and R2 are the ratio of the highest IC_{50} over the lowest IC_{50} taken in the same row or the same column, respectively.
ND, None done.

tions, non linear regression best fitted to a two phase model in each case. The Michaelis–Menten parameters, K_m and V_{max}, are reported in Table 1.

The K_m and V_{max} of the two hydroxylations of AP reported in Table 1 are taken from Kahn *et al.* [9]. The AP concentration used in our experiments was 2 mmol/l which is in the range of the K_m for both reactions.

In order to investigate possible selective effects of MAO inhibitors on the two phases of 7-ECOD,

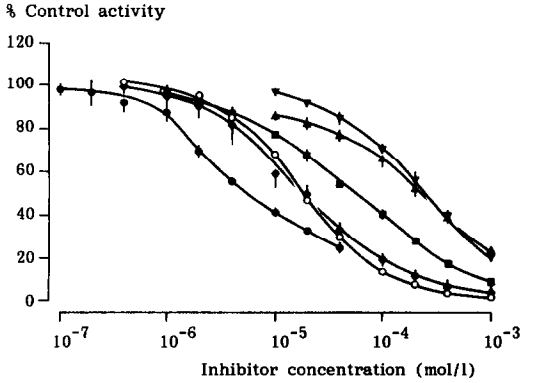

Fig. 1. Inhibition of the hydroxylation on the 3-methyl of antipyrine by (●) clorgyline, (○) phenelzine, (◆) tranylcypromine, (■) l-deprenyl, (▼) pargyline and (▲) iproniazid. Bars indicate SD (N = 4).

incubations were performed in presence of 2 μmol/l and 500 μmol/l of EC. With 2 μmol/l EC, the low K_m activity accounted for 86% of the total activity while it accounted for 8.4% at 500 μmol/l EC.

Bufuralol hydroxylase activity was measured at 10 μmol/l bufuralol, under which condition the low K_m activity component represented 87% of the measured activity.

Inhibition without preincubation

Clorgyline hydrochloride concentration was limited to 100 μmol/l by its solubility in buffer.

Inhibition of BH by l-deprenyl could not be measured because hydroxybufuralol could not be separated from deprenyl during HPLC analysis.

In Fig. 1 the hydroxylation of AP to 3-MeOH-AP is plotted against inhibitors concentrations. From these curves, we graphically estimated the concentrations of inhibitors required to inhibit the reaction by 50% (IC_{50}). Similar curves were obtained for the other reactions. IC_{50} values are listed in Table 2.

For tranylcypromine, an additional experiment was carried out comparing the two enantiomers at 20 μmol/l concentration, towards the low K_m activity of 7-ECOD. The (+) enantiomer appeared to be more potent than the (−), 58% and 41% inhibition respectively (data not shown).

Inhibition after preincubation and washing

In these experiments, the free inhibitor was

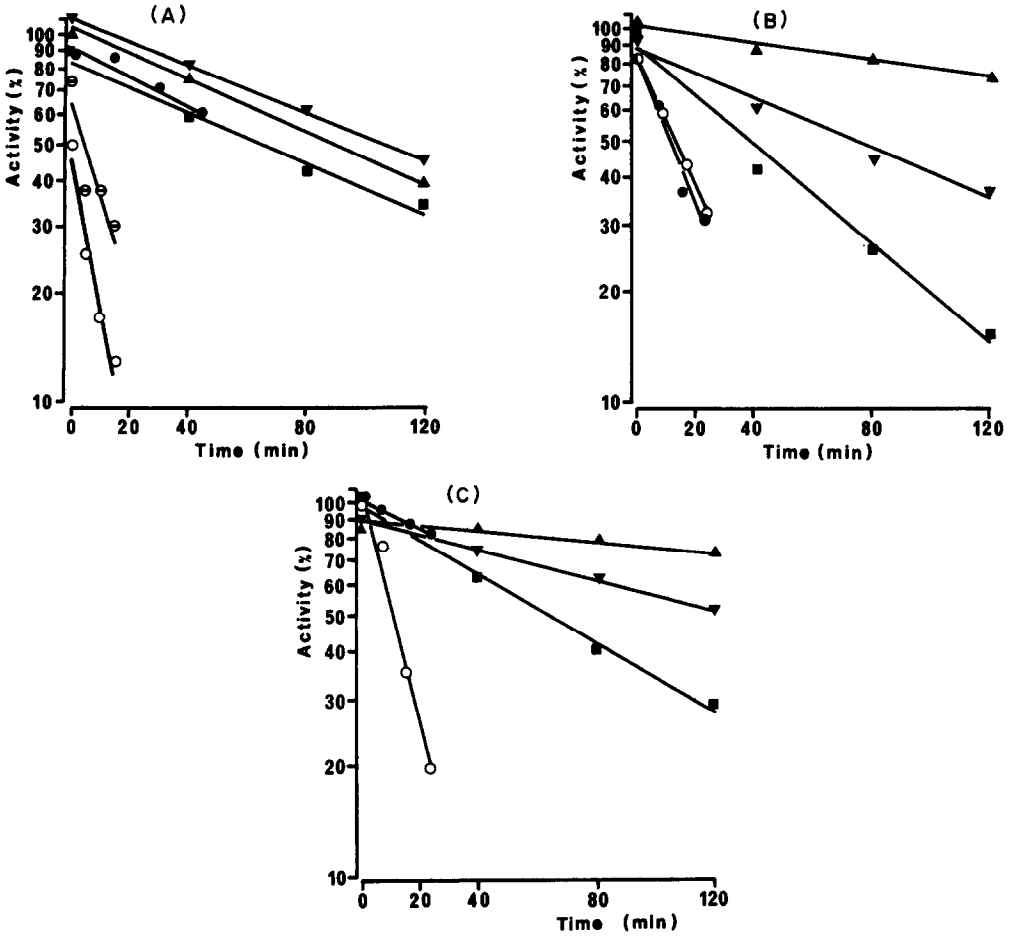

Fig. 2. Time-dependent irreversible inhibition of (A) BH, (B) the low K_m activity of 7-ECOD and (C) the high K_m activity of 7-ECOD by (●) clorgyline 100 μmol/l (A) or 50 μmol/l (B and C), (▼) pargyline 1 mmol/l, (▲) iproniazid 1 mmol/l, (■) l-deprenyl 1 mmol/l, (○,⊖) phenelzine 100 μmol/l. Microsomes were preincubated with the inhibitors over the times indicated. This was carried out in presence of NADPH except for (⊖) phenelzine in A. Following preincubation, the mixture was diluted with chilled buffer then centrifuged. Microsomal pellet was resuspended and assayed for the different activities. For details, see Materials and Methods. No significant loss of activity with time was observed for control samples (without inhibitor added) over this time period. Each point represents the mean of two experiments run in duplicate.

removed, after preincubation with microsomes in absence of substrate, by centrifugation, as described earlier.

In presence of NADPH. In Fig. 2 the activity of the microsomes for BH and 7-ECOD is plotted against the time of preincubation of microsomes with the inhibitors. The activity of the microsomes was stable when incubated alone at 37° for 2 hr. For example, the low K_m activity of 7-ECOD was 26.9 ± 2.6 (SD) pmol/mg/min before preincubation and 24.9 ± 3.1 after 2 hr of preincubation of the microsomes.

Pargyline, iproniazid, deprenyl and tranylcypromine were added at the concentration of 1 mmol/

Table 3. Apparent pseudo-first order rate constants, k'_{app} (sec^{-1}), of time-dependent irreversible inhibitions, in presence of NADPH

Inhibitors and concentrations	Reactions		
	Bufuralol 1'-hydroxylation	7-Ethoxycoumarin *O*-deethylation Low K_m act.	High K_m act.
Iproniazid, 1 mM	$1.4\ 10^{-4}$	$0.44\ 10^{-4}$	$0.28\ 10^{-4}$
Pargyline, 1 mM	$1.2\ 10^{-4}$	$1.3\ 10^{-4}$	$0.76\ 10^{-4}$
l-Deprenyl, 1 mM	$1.3\ 10^{-4}$	$2.5\ 10^{-4}$	$1.7\ 10^{-4}$
Phenelzine, 100 μM	$15\ 10^{-4}$	$6.7\ 10^{-4}$	$12\ 10^{-4}$
Clorgyline, 100, 50 and 50 μM	$1.6\ 10^{-4}$	$7.3\ 10^{-4}$	$1.4\ 10^{-4}$

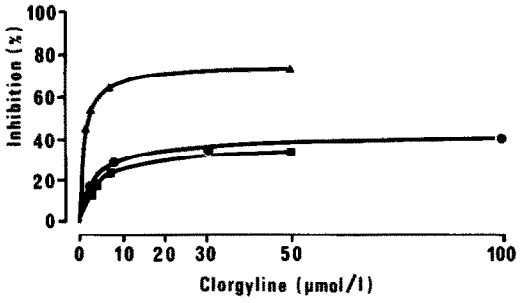

Fig. 3. Effect of clorgyline concentration on irreversible inhibition of (●) BH after 45 min preincubation, (▲) the low K_m activity and (■) the high K_m activity of 7-ECOD after 24 min preincubation. The experimental procedure is the same as in Fig. 2.

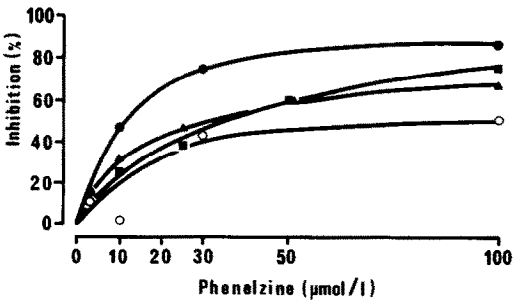

Fig. 4. Effect of phenelzine concentration on irreversible inhibition of BH at zero (○) or 15 min (●) time of preincubation and on (▲) the low K_m activity and (■) the high K_m activity of 7-ECOD after 15 min preincubation. The experimental procedure is the same as in Fig. 2.

l during the preincubation. This high concentration was used in order to minimize a possible depletion of the inhibitor due to microsomal metabolism during the 2-hr preincubation.

Inhibitions by pargyline, iproniazid and deprenyl were time dependent; the inhibition curves could be linearized by a semi-log plot, which is consistent with an apparent first-order kinetics. In each case the correlation coefficient was good (0.9464 to 0.9998, mean 0.9825). Apparent pseudo-first order rate constants (k'_{app}) are reported in Table 3.

Tranylcypromine did not inhibit BH: after a 2-hr preincubation of microsomes with tranylcypromine, BH activity was 123 ± 8% (SD, N = 6) of control. In contrast tranylcypromine slightly inhibited 7-ECOD during the few seconds (less than 10) necessary to mix the microsomes with the inhibitor then to pick-up an aliquot for assay; the activities remaining were 72.7 ± 5.1% (SD, N = 10) and 85.7 ± 2.6% (SD, N = 8) of control for the low K_m and high K_m activities, respectively. This initial inhibition did not increase greatly during the 2 hr of preincubation (65.3 ± 4.5, N = 8 and 79.6 ± 6.0, N = 7 for the low K_m and high K_m activities, respectively). The initial inhibition could not be removed by a second wash, so that it was not due to a residual amount of free inhibitor.

Clorgyline concentration was limited by its solubility in buffer. Nevertheless, as can be seen on Fig. 3, maximal inhibition was seen at 20 μmol/l. Clorgyline 50 μmol/l inhibited 7-ECOD with a marked specificity for the low K_m activity (Figs 2B and 2C).

Phenelzine (100 μmol/l) produced a significant immediate inhibition of BH (50%) (Fig. 2A). The inhibition, measured without or after 15 min preincubation, with phenelzine concentrations ranging from 3 to 100 μmol/l, tended to maximum values at 100 μmol/l (Fig. 4). Inhibition of 7-ECOD by phenelzine was also time dependent, but there was no significant immediate inhibition of the high K_m activity and the low K_m activity was only affected to a small extent under these conditions (Figs 2B and 2C). After 24 min of preincubation, inhibition by 100 μmol/l phenelzine was maximal for the high K_m activity but not for the low K_m activity of 7-ECOD (Fig. 4).

In absence of NADPH. The inhibition observed after preincubation with pargyline, iproniazid, deprenyl and clorgyline were fully or mainly NADPH dependent (Table 4). In contrast, the inhibitory effect of tranylcypromine was NADPH independent. Inhibition of 7-ECOD by phenelzine after preincubation was greatly NADPH dependent.

Table 4. NADPH dependence of irreversible inhibition of microsomal activities by MAOIs

Inhibitors with concentrations and preincubation time	Reactions			
	± NADPH	Bufuralol 1′-hydroxylation	7-Ethoxycoumarin *O*-deethylation Low K_m act.	7-Ethoxycoumarin *O*-deethylation High K_m act.
Iproniazid	+	82.3 ± 9.7	82.1 ± 6.6	75.1 ± 5.3
1 mM, 120 min	−	112 ± 20	101 ± 9	103 ± 9
Pargyline	+	73.7 ± 5.8	42.4 ± 2.1	61.2 ± 2.2
1 mM, 120 min	−	111 ± 13	90.7 ± 7.0	100 ± 5
l-Deprenyl	+	46.9 ± 5.8	24.0 ± 1.4	33.2 ± 2.7
1 mM, 120 min	−	101 ± 10	107 ± 3	104 ± 6
Tranylcypromine	+	123 ± 8	66.1 ± 5.3	78.1 ± 11.3
1 mM, 120 min	−	93.3 ± 9.1	64.2 ± 11.7	69.4 ± 10.7
Phenelzine	+	(see Fig. 2A)	38.6 ± 3.6	18.0 ± 2.6
100 μM, 24 min	−		87.3 ± 8.8	76.8 ± 9.1
Clorgyline	+		33.4 ± 9.4	74.4 ± 4.4
50 μM, 24 min	−	N.D.	90.0 ± 9.7	91.5 ± 5.6

Experimental procedure is the same as in Fig. 2. Values are mean ± SD (N = 4–10) expressed as per cent of control activity (without inhibitor added). N.D. None done.

The immediate inhibition of BH by phenelzine (50%) was only reduced to 25% in absence of the NADPH regenerating system; moreover a rather rapid time dependent inhibition was observed also in absence of NADPH (Fig. 2A).

DISCUSSION

MAOIs were shown to inhibit the cytochrome P-450-dependent metabolism of antipyrine, bufuralol and 7-ethoxycoumarin. These results are consistent with other works reporting inhibition of pethidine *N*-demethylation [4, 8], aminopyrine and dimethylaniline *N*-demethylations, *p*-nitroanisole, *O*-demethylation and aniline hydroxylation [5–7]. In addition, our experiments showed significant irreversible inhibition, with bufuralol and 7-ethoxycoumarin as substrates, particularly by phenelzine.

The IC_{50} values reported in Table 2 indicate that the inhibitors are generally not very specific (see R1 values in Table 2), so it may be expected that MAOIs interact with a broad range of substrates. In particular, the two hydroxylations of AP were inhibited by similar concentrations of MAOIs in spite of the assumption that the two reactions are catalysed by two isozymes. Indeed, it has been shown that these two metabolic pathways are selectively inducible [15].

In contrast, inhibitory potencies are very variable (see R2 values in Table 2). It is important to emphasize that in spite of similarities in chemical structures, clorgyline was much more potent than the two other acetylenic MAOIs, pargyline and deprenyl. The same observation can be made for iproniazid and pargyline, both hydrazine derivatives, the second one being much more potent than the first.

From the IC_{50} values in Table 2, clorgyline appeared to be the most potent inhibitor with IC_{50} values in the micromolar range, while toloxatone, the only reversible MAOI among the compounds studied, was the least potent inhibitor. Data from the experiments designed to study the reversibility of the inhibitions by MAOIs are reported in Fig. 2. Except for tranylcypromine, the MAOIs produced time dependent irreversible inhibitions consistent with an apparent first-order kinetics; this suggests that the inhibitors were not depleted to any significant extent during the time-courses of the preincubations, but we cannot rule out the possibility of a metabolite being an inhibitor.

The mechanism of irreversible inhibition is not clearly defined, but the presence of NADPH was found to be necessary for the inhibitory process in all cases except for phenelzine and tranylcypromine (Table 4). This, and the time dependence of the process, suggest that the mechanism of inhibition requires an enzymatic step, which could be catalysed by the cytochrome P-450 itself. Irreversible inhibition by tranylcypromine was neither NADPH-dependent (Table 4) nor time-dependent (see Results), and is consistent with a direct, chemical interaction of the inhibitor with the enzyme. The mechanism of the irreversible inhibition by phenelzine is less clear. Indeed there was an immediate inhibition of the bufuralol hydroxylation, which partly required the presence of NADPH. Moreover, in the absence of NADPH, there was still a time-dependent inhibition (Fig. 2A), while 7-ECOD inhibition mainly depended on the presence of NADPH (Table 4). Phenelzine has been reported to irreversibly inactivate cytochrome P-450 by a time- and NADPH-dependent process [16], and this may account for the NADPH-dependent inhibition of 7-ECOD. However, our result about a non-NADPH-dependent inhibition of BH is consistent with the findings of Ortiz de Montellano *et al.* [17], who reported a time-dependent loss of cytochrome P-450 when incubated with phenelzine, even without NADPH.

IC_{50} values are high compared with plasma concentrations of MAOIs: following a single oral dose of 30 mg phenelzine [18] or 20 mg tranylcypromine [19] or 200 mg toloxatone [20] in humans, peak plasma concentrations of these compounds are about 0.1, 1.0 and 8.8 μmol/l respectively, although presystemic blood or liver concentrations following the absorption phase might be higher. However, the significant irreversible inhibitions observed, particularly with phenelzine and clorgyline, might produce inhibition of cytochrome P-450 dependent metabolism during a therapeutic treatment because the effect is cumulative when repeated dosing.

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