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EFFECT OF MONOAMINE OXIDASE INHIBITORS ON THE *N*-DEMETHYLATION AND HYDROLYSIS OF MEPERIDINE*

N. R. EADE and K. W. RENTON

Department of Pharmacology and Therapeutics, and Department of Pediatrics, McGill University, Montreal 109, Canada

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Abstract—The administration of meperidine to patients being treated with a monoamine oxidase inhibitor (MAOI) can produce lethal reactions of unknown etiology. Inhibition of microsomal degradation of meperidine has been suggested as the explanation of these reactions. In this study the kinetics of the inhibition of *N*-demethylation and hydrolysis of meperidine by six MAOI and SKF-525A are described. The hydrazine MAOI, phenelzine and iproniazid, *d*- and *l*-amphetamine and SKF-525A, were competitive inhibitors of meperidine *N*-demethylation, while pargyline and tranylcypromine were noncompetitive inhibitors. The hydrolysis of meperidine was inhibited only by pargyline and the inhibition was competitive. The possibility that inhibition of both *N*-demethylation and hydrolysis of meperidine may be involved in the etiology of the adverse reactions which occur between the MAOI and meperidine is discussed.

MEPERIDINE is metabolized in the liver of rats,[1] dogs and humans[2] by the two pathways shown in Fig. 1. Inhibition of one or more of these pathways was postulated as the mechanism by which the monoamine oxidase inhibitors (MAOI) produced severe toxic interactions when these drugs were given in combination with meperidine,[3,4] since it had previously been shown that MAOI inhibited the degradation of drugs such

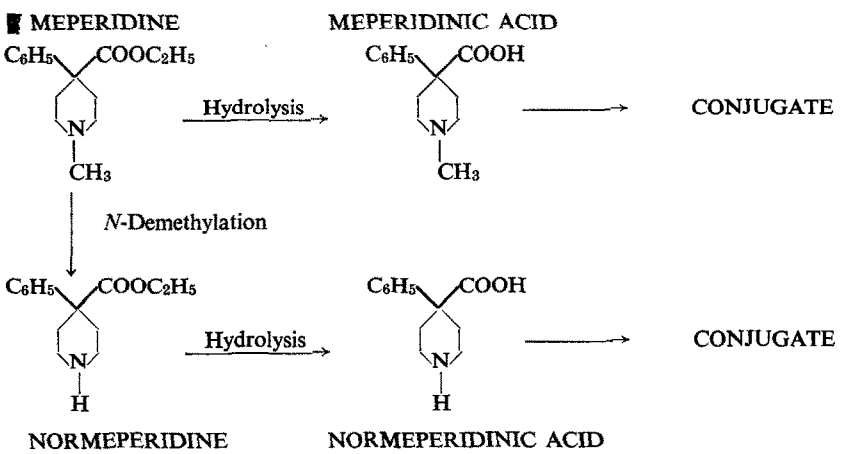

FIG. 1. Two pathways of meperidine metabolism.

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as hexobarbital[5,6] ethylmorphine[7] and aminopyrine.[8] Recently Clark[9] showed that phenelzine inhibited the *N*-demethylation of meperidine, but had no effect on its hydrolysis, and Eade and Renton[10] demonstrated that both phenelzine and tranylcypromine markedly inhibited the rate of disappearance of meperidine from the mouse.

In an effort to clarify this interaction, a number of compounds were studied with respect to their ability to inhibit the *N*-demethylation and the hydrolysis of meperidine. They included phenelzine and iproniazid (hydrazines), tranylcypromine (a cyclic primary amine) and pargyline (a tertiary amine), all of which compounds have been reported to be potent inhibitors of monoamine oxidase (MAO).[11] The *d*- and *l*-isomers of amphetamine (primary amines), reportedly very weak inhibitors of MAO,[12] were also included as was SKF-525A, a potent and extensively studied inhibitor of microsomal enzymes.[13]

METHODS

Male Sprague–Dawley rats (200–300 g) obtained from Canadian Breeding Farms, St. Constant, Quebec, were used in these experiments. The rats were killed by a blow on the head, bled out, and the livers removed immediately, weighed and kept in an ice bath.

Microsomes. The microsomal fraction was prepared by the method of Kato *et al.*[14] Using a homogenizer with a Teflon plunger, the livers were homogenized in 4 vol. by weight of 1·5% potassium chloride solution. The homogenate was centrifuged at 9000 *g* for 10 min in a refrigerated centrifuge and the supernatant (microsomal and soluble cytoplasmic fraction) was carefully removed and the residue discarded. The supernatants from several livers were pooled and freeze-dried in 10-ml portions. When stored at −10°, they were stable for at least 3 months. When required, reconstituted supernatant containing the microsomal and soluble cytoplasmic fraction was prepared by the addition of distilled water to the original volume. The activity of the reconstituted fractions was similar to that of freshly prepared microsomes. For example, enzyme batch no. 1 (see Table 1) retained 95·2 per cent of its activity after storage for 5 weeks at −10°.

Protein determination. The percentage protein in the freeze-dried preparations was determined by the biuret method.[15]

Meperidine N-*demethylase.* The *N*-demethylation of meperidine was measured by determining the amount of formaldehyde formed. The reaction mixture was a modification of that described by Axelrod,[1] and contained 0·1 ml of reconstituted enzyme preparation, 25 μmoles $MgCl_2$, 0·25 μmole $NADP^+$, 10 μmoles glucose 6-phosphate, 150 μmoles phosphate buffer (pH 7·2), 25 μmoles of neutralized semicarbazide HCl, and 50 μmoles nicotinamide. This concentration of nicotinamide has been reported to inhibit the *N*-demethylation of aminopyrine,[16] but in our experiments concentrations up to 100 μmoles had no such effect. Meperidine and MAOI were added to provide a range of concentrations and the reaction mixture was made up to a final volume of 2 ml with distilled water. Samples were incubated for 30 min at 37° in a Dubnoff shaking water bath. The reaction was linear over this period of time and was terminated by the addition of 0·5 ml of 5% zinc sulfate followed by 0·5 ml of 0·3 N barium hydroxide and mixing. After centrifugation, the clear supernatant was used for formaldehyde assay. Formaldehyde was determined by the method of Cochin and Axelrod[17] in which 1·5 ml supernatant was added to 1 ml of Nash reagent and the

mixture incubated for 30 min at 60°. After cooling to room temperature, the tubes were read at 415 mμ in a spectrophotometer. Enzyme activity was expressed as micrograms of formaldehyde formed per hour per gram of protein.

Meperidine esterase. Meperidine hydrolysis was measured by determining the amount of ethanol formed.[9] The reaction mixture contained 0·4 ml of reconstituted microsomal fraction, 6 μmoles phosphate buffer (pH 7·3), meperidine, and one of six MAOI or SKF-525A in a total volume of 1·0 ml. In the first series of experiments, which served as a screen for inhibitory activity, meperidine was kept constant at 1 μmole and the MAOI was present at 10 or 40 μmoles. In subsequent experiments with pargyline, the meperidine concentration varied from 0·5 to 5 μmoles and pargyline varied from 0·1 to 2·5 μmoles. All samples were incubated for 30 min at 37° in a Dubnoff shaking water bath, the reaction being linear over this period of time. The reaction was terminated by the addition of 0·5 ml of 70% perchloric acid. The samples were cooled in an ice bath for 10 min and, after brief centrifugation, 1 ml of 1·1 M potassium phosphate (pH 8·2) was added and mixed with the supernatant without disturbing the sediment. This mixture was recentrifuged and 1 ml of clear supernatant was added to 2 ml of 0·032 M pyrophosphate buffer (pH 8·8), 2·5 mM NAD^+, and 50 units of yeast alcohol dehydrogenase. The mixture was incubated for 30 min at 25° in a water bath and the samples were read in a spectrophotometer at 340 mμ. Enzyme activity was expressed as micrograms of ethanol formed per hour per gram of protein.

Statistics. Kinetic data were plotted according to the method of Lineweaver and Burk.[18] Each point was the mean of six determinations and the best-fitting straight line was determined by the method of least squares. Michaelis–Menten constants, K_m and V_{max}, were determined by the method of Wilkinson,[19] utilizing an IBM 360-75 digital computer. The program used was a modification of the FORTRAN program published by Cleland.[20]

RESULTS

Meperidine demethylase. As shown in Figs. 2 and 3, each of the six MAOI studied in this series and SKF-525A inhibited the *N*-demethylation of meperidine to normeperidine. The results have been plotted in Fig. 2 by the method of Lineweaver and Burk.[18] The inhibition produced by phenelzine, iproniazid, *d*-amphetamine, *l*-amphetamine and SKF-525A was competitive, as indicated by a constant V_{max} and increasing K_m (Table 1).

Pargyline and tranylcypromine also showed inhibition, as indicated by a decrease in the amount of formaldehyde formed. This inhibition, as illustrated by the Lineweaver–Burk plot in Fig. 3, did not resemble the classical plot for competitive inhibition and suggests inhibition of a noncompetitive nature.

Meperidine esterase. As shown in Table 2, of the seven compounds known to be inhibitors of monoamine oxidase which were tested for their ability to inhibit the hydrolysis of meperidine to meperidinic acid, only pargyline inhibited this reaction, as demonstrated by a reduction in the amount of ethanol formed ($P < 0.05$). Both concentrations of SKF-525A and the 40 mM concentration of iproniazid also appeared to be active, but a precipitate appeared in the reaction mixture and these compounds were therefore excluded from further study.

The effect of pargyline (0·1 to 2·5 mM) on the rate of hydrolysis of a range of concentrations of meperidine was also studied, and the results have been plotted by the

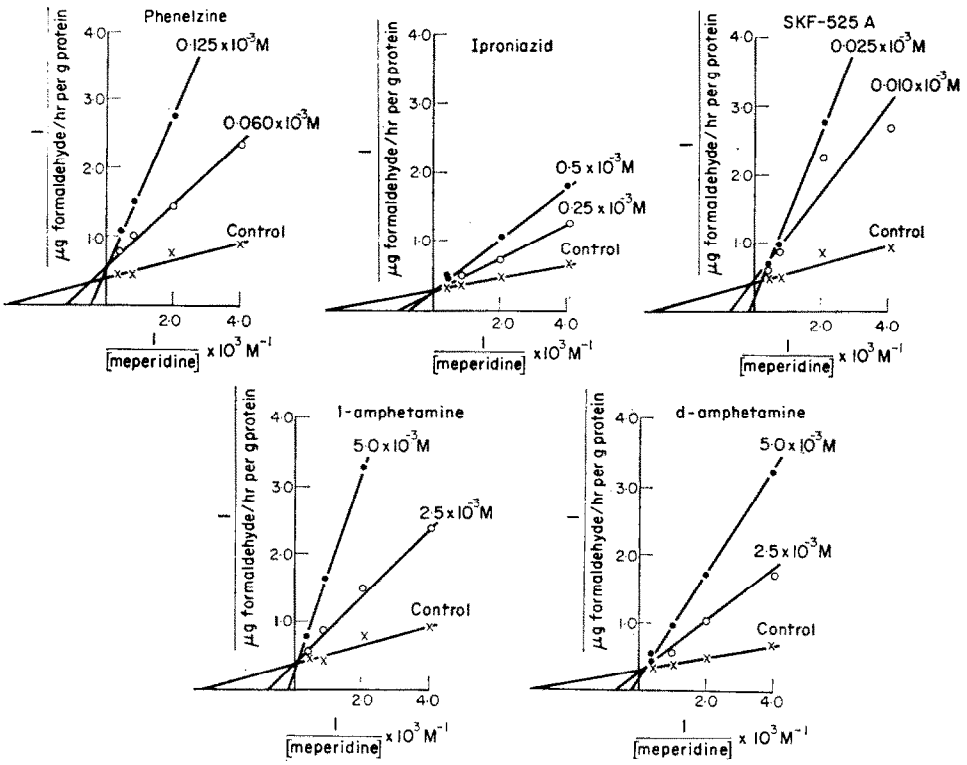

FIG. 2. Competitive inhibition of meperidine *N*-demethylation by monoamine oxidase inhibitors and SKF-525A. Incubation as described under Methods. Lineweaver–Burk plot of meperidine concentration versus rate of *N*-demethylation with varying concentrations of phenelzine, iproniazid, SKF-525A, *d*- and *l*-amphetamine.

TABLE 1. EFFECT OF MAOI ON MEPERIDINE *N*-DEMETHYLATION

Inhibitor	conc. (mM)	$K_m \times 10^{-3}$ M*	V_{max}* (μg HCHO/hr/g of protein)
Control	(1)	0·239 ± 0·019	2·923 ± 0·057
Control	(2)	0·385 ± 0·127	2·559 ± 0·252
Phenelzine	(2) 0·060	0·488 ± 0·063	1·353 ± 0·057†
	0·125	2·108 ± 0·344†	1·796 ± 0·160
Iproniazid	(1) 0·25	0·732 ± 0·082†	3·219 ± 0·137
	0·50	1·120 ± 0·241†	2·769 ± 0·597
SKF-525A	(2) 0·010	3·619 ± 1·496†	4·308 ± 1·185
	0·025	—	—
l-Amphetamine	(2) 2·5	1·691 ± 0·524†	2·818 ± 0·452
	5·0	1·582 ± 0·072†	8·332 ± 0·538†
d-Amphetamine	(1) 2·5	1·227 ± 0·057†	3·224 ± 0·169
	5·0	2·030 ± 1·621†	3·451 ± 1·400
Pargyline	(1) 0·5	0·389 ± 0·032	1·569 ± 0·039†
	1·0	0·824 ± 0·163	1·296 ± 0·102†
Tranylcypromine	(2) 5·0	0·464 ± 0·068	1·303 ± 0·062†
	10·0	0·308 ± 0·052	0·788 ± 0·011†

* K_m and V_{max} were determined by the method of Wilkinson.[19]
† Significantly different from control at $P < 0·05$.
Figures in parentheses indicate the enzyme batch number. SKF-525A data (0·025 mM) were not adequate for statistical analysis.

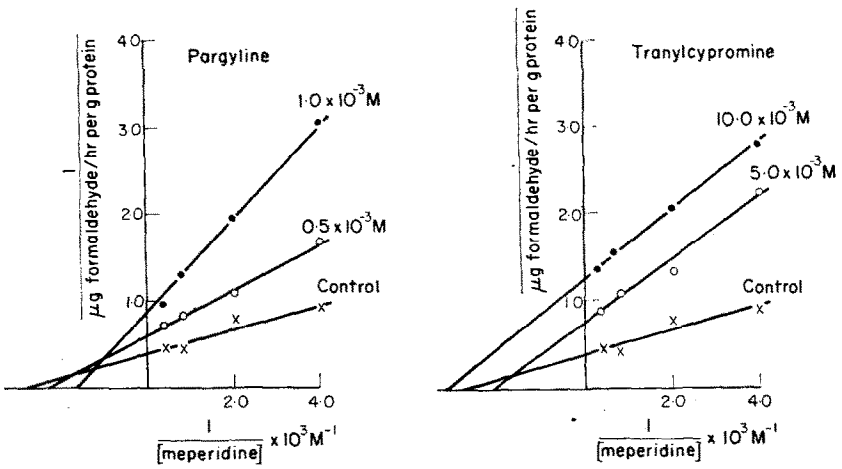

FIG. 3. Inhibition of meperidine *N*-demethylation by pargyline and tranylcypromine. Incubation as described under Methods. Lineweaver-Burk plot of meperidine concentration versus rate of *N*-demethylation with varying concentrations of pargyline and tranylcypromine.

TABLE 2. EFFECT OF MAOI ON THE AMOUNT OF ETHANOL FORMED BY THE HYDROLYSIS OF 1μmole MEPERIDINE TO MEPERIDINIC ACID AND ETHANOL*

MAOI	Conc. (mM)	Ethanol formed (μg/hr/g protein)
Control		80·2 ± 7·1
Phenelzine	10	94·2 ± 4·9
	40	84·2 ± 14·1
Iproniazid	10	79·8 ± 4·1
	40	51·0 ± 6·9†
Tranylcypromine	10	76·5 ± 3·8
	40	69·8 ± 4·6
Pargyline	10	40·8 ± 3·8†
	40	34·4 ± 4·9†
d-Amphetamine	10	79·8 ± 5·7
	40	75·4 ± 9·2
l-Amphetamine	10	86·4 ± 12·2
	40	74·3 ± 5·8
SKF-525A	10	68·8 ± 5·9
	40	39·9 ± 5·7†

* Results are expressed as mean ± S.E.; N = 6.
† Student *t*-test; significantly different from control at $P < 0.05$.

TABLE 3. EFFECT OF PARGYLINE ON MEPERIDINE HYDROLYSIS

Inhibitor conc. (Pargyline) (mM)	$K_m \times 10^{-3}$ M*	V_{max}* (μg ethanol/hr/g of protein)
Control	1·097 ± 0·477	303·8 ± 47·6
0·1	1·218 ± 0·278	255·0 ± 20·9
0·5	2·634 ± 0·731†	263·0 ± 27·2
2·5	9·042 ± 0·617†	296·5 ± 30·1

* K_m and V_{max} were determined by the method of Wilkinson.[19]
† Significantly different from control at $P < 0.05$.

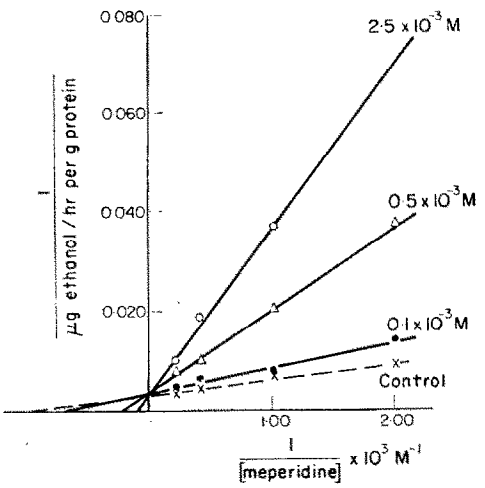

FIG. 4. Competitive inhibition of meperidine hydrolysis by pargyline.Incubation as described under Methods. Lineweaver–Burk plot of meperidine concentration versus rate of hydrolysis with varying concentrations of pargyline.

method of Lineweaver and Burk[18] in Fig. 4. Inhibition produced by pargyline was reversed by increasing the substrate concentration. With increasing inhibitor concentrations, the V_{max} was relatively constant while the K_m increased (Table 3), indicating that pargyline was a competitive inhibitor of meperidine hydrolysis.

DISCUSSION

The hydrazine MAOI, iproniazid and phenelzine, were shown to be competitive inhibitors of meperidine *N*-demethylation. These results are supported by the work of Clark,[9] who also showed that phenelzine was a competitive inhibitor of this enzyme system. He postulated that the inhibition occurred at the terminal stage of the electron transport pathway detailed by Omura *et al.*,[21] at the site where the active oxygen donor (cytochrome P_{450}.0) oxidized meperidine to normeperidine. The competitive nature of this inhibition was also reflected in the experiments of Eade and Renton,[10] who showed that phenelzine *in vivo* inhibited the degradation of meperidine in the mouse, this inhibition being readily reversible and related to phenelzine serum levels. The competitive inhibition demonstrated by these two hydrazines for this microsomal enzyme is in marked contrast to the irreversible inhibition these agents produce with monoamine oxidase.[11] *d*- And *l*-amphetamine and SKF-525A were also competitive inhibitors of meperidine *N*-demethylation.

In our experiments, tranylcypromine and pargyline also inhibited the *N*-demethylation of meperidine; however, the inhibition did not conform to Michaelis–Menten kinetics for competitive inhibition and suggested non-competitive inhibition.

With respect to the action of these compounds on meperidine esterase, only pargyline unequivocally inhibited the hydrolysis reaction. This inhibition was competitive for the inhibitor concentration range of 0·1 to 2·5 mM. Iproniazid and SKF-525A, which may have some inhibitory activity with respect to this enzyme, were discounted in this study because of the formation of a precipitate in the reaction mixture.

It has been suggested that the adverse reactions which have been reported after the combined administration of the MAOI and meperidine were due to inhibition of meperidine degradation.[3,4] This is supported by the crude observation that in mice the symptoms evoked by the combined administration of MAOI and meperidine are similar to those produced by large doses of meperidine alone.[10] The results reported here would support such a hypothesis and indicate that *N*-demethylation is probably the primary pathway inhibited. However, *d*- and *l*-amphetamine also inhibited *N*-demethylation of meperidine as did the hydrazine MAOI and to our knowledge no adverse reactions have been reported for the combination of amphetamines and meperidine, which one might expect if inhibition of *N*-demethylation were the sole mechanism responsible. It is probable that another mechanism, as yet undetermined, is concerned in this toxic interaction and that it is not wholly dependent on impaired metabolism and accumulation of meperidine. This hypothesis is similar to that postulated by Jounella,[22] and by Loveless and Maxwell.[23]

More recently, Rogers and Thornton[24] observed that the increased toxicity of analgesics combined with MAOI correlated well with increased 5-hydroxytryptamine levels in the mouse brain and suggested a critical level of this amine might be necessary for the production of an adverse reaction. These authors discounted impaired metabolism of the analgesic as a contributing factor, because in their experiments tranylcypromine had no effect on the metabolism of pentazocine. They also suggested that the MAOI did not have to be present at the time the adverse reaction occurred, because in clinical reports it had been stated that some patients had an adverse reaction 36 hr after their last dose of MAOI, a time when it was presumed that most of the MAOI had been metabolized and excreted. However, Evans *et al.*[25] have demonstrated that humans can be phenotyped into fast and slow acetylators of phenelzine and it is possible that patients reacting late belonged to a slow-acetylating group and that phenelzine was still present when these reactions occurred.

In summary, it has been shown that the MAOI, phenelzine, iproniazid, tranylcypromine and pargyline, the amphetamines and SKF-525A inhibited the *N*-demethylation of meperidine, and pargyline inhibited its hydrolysis. This inhibition may contribute to the adverse reactions seen in man, although it is unlikely to be the complete explanation.

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