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DEAMINATION OF β-PHENYLETHYLAMINE BY MONOAMINE OXIDASE—INHIBITION BY IMIPRAMINE*

JEROME A. ROTH and C. N. GILLIS

From the Departments of Anesthesiology and Pharmacology, Yale University School of Medicine, New Haven, Conn. 06520, U.S.A.

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Abstract—The oxidative deamination of tyramine (Tyr), 5-hydroxytryptamine (5-HT), and β-phenylethylamine (PEA) by mitochondrial preparations of rabbit lung and brain was inhibited by imipramine. This tricyclic iminodibenzyl antidepressant drug was most effective in decreasing the deamination of PEA: at 1×10^{-4}M imipramine, deamination of PEA, Tyr and 5-HT was inhibited by approximately 70, 45 and 45 per cent, respectively, when either lung or brain mitochondrial monoamine oxidase (MAO) preparations were used. Imipramine-induced inhibition of MAO was shown to be of a mixed type based on Lineweaver–Burk plots, but was found to be completely reversible. The desmethyl and didesmethyl derivatives of imipramine were equally as effective as the parent drug in inhibiting the deamination of PEA, whereas the *N*-oxide analog of imipramine was less effective as an inhibitor of this reaction. These results support the premise that the action of imipramine as a clinically effective antidepressive agent may be related to its inhibitory effect on the specific form of MAO which deaminates PEA.

IT HAS BEEN suggested that the deficit of endogenous β-phenylethylamine (PEA) may be one of the biochemical lesions in depression.[1,2] Fischer *et al.*[3] have shown that antidepressant drugs, including monoamine oxidase (MAO) inhibitors and tricyclic iminodibenzyl derivatives, elevate the concentration of PEA in human urine and in rat brain, though the mechanism by which tricyclic antidepressant drugs increase PEA levels in the body is not known.[3] While inhibition of the oxidative deamination of PEA could account for this increase, it is often stated that iminodibenzyl drugs, for example imipramine, have little effect on the activity of mitochondrial monoamine oxidase.[4,5]

Several reports, however, challenge the proposal that tricyclic antidepressants do not affect MAO activity. Thus, Gabay and Valcourt[6] indicated that imipramine effectively inhibits the deamination of kynuramine by purified preparation of rabbit liver mitochondrial MAO. Similarly, Halaris *et al.*[7] found that chlorimipramine inhibits deamination of 5-hydroxytryptamine (5-HT). In agreement with these findings, we have reported[8] that the imipramine analog, desmethylimipramine, inhibited the deamination not only of 5-HT but also of norepinephrine (NE), epinephrine, tyramine and dopamine. However, the effect of tricyclic antidepressant drugs on the oxidative deamination of PEA remains to be determined.

Recently, it was reported that PEA is metabolized by a different form of MAO from that which deaminates 5-HT or NE.[9] The type B form of the oxidase, which

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deaminates PEA, is selectively inhibited in some species by pargyline and Deprenyl, whereas the type A form of MAO, which is responsible for 5-HT and NE deamination, is inhibited by harmine and related compounds.[10] It is not known whether imipramine displays a selective inhibitory action toward either type A or type B MAO. The purpose of the present study was to determine the extent to which the tricyclic antidepressant drug, imipramine, as well as some of the metabolic derivatives, influences oxidative deamination of PEA by type B MAO.

MATERIALS AND METHODS

Male albino rabbits weighing approximately 2 kg were fed *ad lib.* on standard laboratory chow. The following procedure was used to prepare lung mitochondria free of contamination by platelet and plasma MAO. Rabbits were given i.v. injections, in succession, of herapin (500 I.U./kg) and a mixture of Dial (30 mg/kg) and urethane (120 mg/kg). The lungs were removed from the animals, placed in a polyethlene chamber within a water bath maintained at 37° and were perfused through a pulmonary arterial cannula for approximately 5 min with Krebs medium.[11] Areas of lung which were poorly perfused were discarded and the remaining portions were homogenized in 2 vol. (by weight) of 0·1 M potassium phosphate buffer, pH 7·4, containing 0·25 M sucrose in a Waring blender (5 sec; two times) and then in a motor-driven Teflon–glass homogenizer. The resulting homogenate was centrifuged twice at 600 *g* for 10 min to remove cellular debris. The supernatant solution from the second centrifugation was recentrifuged at 10,000 *g* for 20 min and the resulting mitochondrial precipitate was resuspended by homogenization in the phosphate buffer described above. This suspension was recentrifuged at 10,000 *g* for 20 min and the final mitochondrial precipitate was resuspended by homogenization in 0·1 M potassium phosphate buffer, pH 7·4. This preparation of MAO was stored frozen for periods up to 3 months with only a minimal loss of activity. Rabbit brain mitochondria were isolated in a similar manner except that homogenization in the Waring blender was omitted.

Phenylethylamine deamination was assayed in the following manner: 1·8 μM 1-^{14}C-β-phenylethylamine-HCl was incubated with either lung or brain mitochondria at 37° for 5 min, unless indicated otherwise, in the presence of 0·05 M potassium phosphate buffer, pH 7·4. Varying amounts of imipramine or imipramine analogs were added to the reaction mixtures (total volume, 2 ml). Reactions were terminated by the addition of 2 ml of 0·4 M perchloric acid and the resulting precipitated protein was removed by centrifugation at 3000 *g* for 10 min. Three-ml aliquots of the supernatant solutions were removed, adjusted to pH 6 with KOH, and passed over a Bio Rex-70 column (approximately 3 × 0·6 cm; sodium form, pH 6·0). The products, phenylacetic acid (PAA) or/and aldehyde, formed in the reactions were eluted from the columns with H_2O. A total effluent volume of 8 ml was collected and a 0·5-ml aliquot was removed, added to 10 ml of Bray's solution[12] and radioactivity was determined in a scintillation spectrometer (Packard, model 3320).

Initial studies indicated that all of the radioactive deaminated products had eluted in the first 8 ml of the H_2O wash. Evidence that the ^{14}C-material eluting in this H_2O fraction was formed from MAO deamination of PEA is provided by the fact that when incubations were conducted in the presence of pargyline (10^{-3}M), radioactivity in this fraction was decreased to levels equivalent to those of "no enzyme" control

incubations. The remaining ^{14}C-material bound to the column was removed by elution with 0·25 M HCl. Over 90 per cent of the total radioactive material placed over the column could be recovered by this procedure.

The deamination of ^{14}C-5-HT and ^{14}C-tyramine was determined as described above, except that reactions were allowed to proceed at 37° for either 15 or 10 min respectively. Blank values for 5-HT deamination were determined by incubating the reaction mixtures in the presence of the MAO inhibitor, harmaline.

The nature of imipramine binding to type A and type B MAO was determined in the following manner. Lung mitochondrial preparations were divided into two equal volumes, and imipramine in H_2O (10^{-3}M, final concentration) was added to one fraction and an equivalent amount of H_2O was added to the other fraction. An aliquot (2 ml) of each solution was removed and 0·1-ml samples were assayed for PEA and 5-HT deaminating activity as described above. In the case of PEA deamination, the 2-ml aliquots were first diluted with 0·5 ml buffer. The remaining mitochondrial suspensions were centrifuged at 27,000 *g* for 15 min and the resulting precipitates were resuspended by homogenization in buffer. The latter two steps were repeated three times and the final, washed and resuspended, mitochondrial preparations were assayed (0·1-ml aliquots) for PEA and 5-HT deaminating activity as described above.

1-^{14}C-β-phenylethylamine-HCl (7 mCi/m-mole) was purchased from New England Nuclear Corp., Boston, Mass.; 1-^{14}C-tyramine-HCl (42 mCi/m-mole) and 3-^{14}C-5-hydroxytryptamine creatinine sulfate (56–58 mCi/m-mole) were purchased from Amersham/Searle Corp., Arlington Heights, Ill. Imipramine-HCl, desmethylimipramine-HCl, didesmethylimipramine-HCl, and imipramine *N*-oxide were gifts from the CIBA-GEIGY Corp., Summit, N.J. Harmaline was obtained from Aldrich Chemical Co., Inc., Milwaukee, Wis.

RESULTS

In order to establish that the lung preparations used in these studies contained both type A and type B forms of MAO, harmaline, a specific inhibitor of type A MAO, was used to inhibit the deamination of 5-HT and PEA.[10] At a concentration of 5×10^{-7} M harmaline, deamination of 5-HT was completely abolished, whereas deamination of PEA was unaffected (8 per cent inhibition). These results are consistent with previous data[10] indicating that harmaline is a specific inhibitor of rabbit type A MAO as measured by 5-HT deamination and that PEA is metabolized by the type B form of MAO, which is relatively insensitive to inhibition by harmaline.

Initial studies were designed to examine the effect of imipramine on MAO activity when tyramine was used as a mixed type A and type B MAO substrate. As can be seen in Fig. 1, imipramine inhibits deamination of tyramine by mitochondrial preparations of rabbit lungs and brains. Oxidation of tyramine is inhibited approximately 50 per cent by 10^{-4}M imipramine. At drug concentrations around 10^{-3}M, the reaction is inhibited greater than 80 per cent, and at drug concentrations below 3×10^{-5}M, less than 20 per cent deamination of tyramine is observed. Furthermore, the results presented in Fig. 1, indicating that tyramine deamination is essentially completely inhibited by imipramine, suggest that both the type A and type B forms of MAO are inhibited by this antidepressant drug.

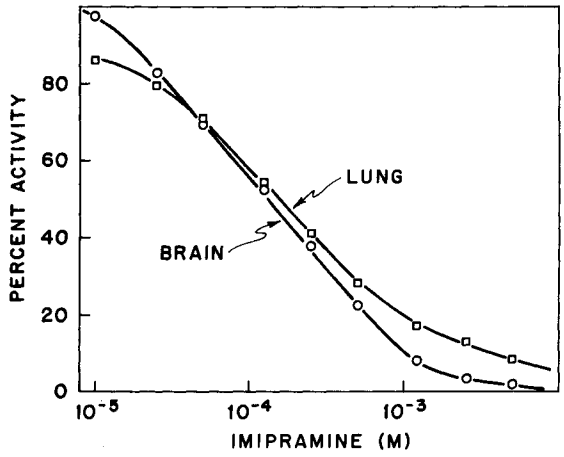

FIG. 1. Effect of imipramine on deamination of tyramine by lung or brain monoamine oxidase (MAO). ^{14}C-tyramine, 1·2 μM, in the presence of varying amounts of imipramine was incubated with either brain (5·2 mg protein) or lung (2·3 mg protein) mitochondria in 0·05 M buffer for 10 min at 37°. Deaminated product formed was assayed according to methods described in the text. In the absence of imipramine, 0·36 nmole product was produced.

Previous work in this laboratory[8] indicated that deamination of the type A MAO substrate, 5-HT, by crude preparations of lung and brain MAO was effectively inhibited by both imipramine and desmethylimipramine. The action of imipramine on deamination of the type B mitochondrial MAO substrate, PEA, is illustrated in Fig. 2. Approximately 20 per cent inhibition of both brain and lung mitochondrial type B MAO activity is observed at a drug concentration of 5×10^{-6}M. At 3×10^{-5}M imipramine, approximately 50 per cent inhibition of PEA deamination occurs, and at 5×10^{-4}M, greater than 85 per cent inhibition is achieved.

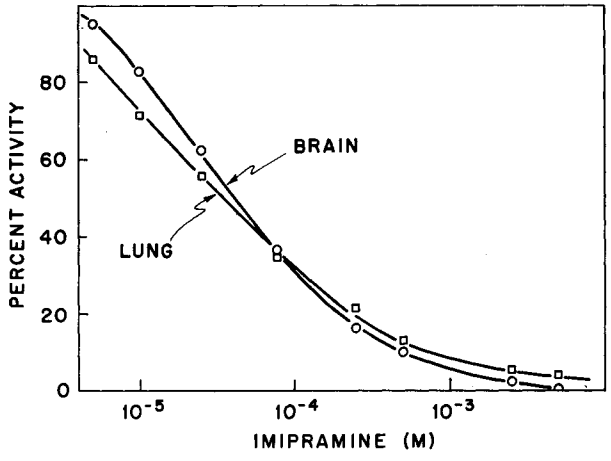

FIG. 2. Effect of imipramine on deamination of PEA by lung and brain monoamine oxidase (MAO). ^{14}C-PEA, 1·8 μM, in the presence of varying amounts of imipramine was incubated with either brain (5·2 mg protein) or lung (1·2 mg protein) mitochondria in 0·05 M buffer for 5 min at 37°. Deaminated product formed was assayed according to methods described in the text. In the absence of imipramine, 2·42 nmoles product was produced.

TABLE 1. COMPARATIVE EFFECT OF IMIPRAMINE ON THE DEAMINATION OF PEA AND 5-HT BY BRAIN MITOCHONDRIAL MONOAMINE OXIDASE*

Imipramine concn (M)	Amount of product formed: PAA (nmoles) 1	PAA (nmoles) 2	5-HIAA (nmoles) 1	5-HIAA (nmoles) 2	Average % inhibition: PAA	Average % inhibition: 5-HIAA
None	1·624	1·440	0·390	0·544		
$2·5 \times 10^{-4}$	0·233	0·178	0·107	0·159	86·7	71·8
$1·0 \times 10^{-4}$	0·410	0·367	0·208	0·267	74·6	48·6
$1·0 \times 10^{-5}$	1·222	1·163	0·350	0·474	22·0	11·7
$5·0 \times 10^{-6}$	1·586	1·315	0·346	0·539	5·3	6·2

* Reaction mixtures containing either 3·6 nmoles PEA or 5-HT, 5·2 mg protein and varying amounts of imipramine, as indicated, in a total of 2 ml buffer, were incubated at 37° for 5 min when PEA was substrate and for 15 min when 5-HT was substrate.

The ability of imipramine to inhibit deamination of both 5-HT and PEA is compared in Table 1 for brain mitochondrial MAO and in Table 2 for lung mitochondrial MAO. With both brain and lung preparations of MAO, PEA deamination is more effectively inhibited by the tricyclic drug than is that of 5-HT. Also, it should be noted that the effects of imipramine on brain or lung mitochondrial deamination of either 5-HT or PEA are similar (Tables 1 and 2).

The effectiveness of iproniazid as an inhibitor of MAO has been found to be enhanced by preincubating the enzyme in the presence of this drug.[13] Accordingly, we studied the effect of preincubating lung and brain preparations of MAO for 5 min with the tricyclic antidepressant drug. These experiments indicate that preincubation with imipramine (5×10^{-5}M) did not alter the extent of PEA deamination. At a concentration of 3·6 μM PEA, the control and preincubated reactions were inhibited by 45·2 and 48·6 per cent, respectively, and at 35·7 μM PEA, both reactions were inhibited by 38·7 per cent. Similar results were obtained when 5-HT was used as a substrate.

TABLE 2. COMPARATIVE EFFECT OF IMIPRAMINE ON THE DEAMINATION OF PEA AND 5-HT WITH LUNG MITOCHONDRIAL MONOAMINE OXIDASE*

Imipramine concn (M)	Amount of product formed: PAA (nmoles) 1	PAA (nmoles) 2	5-HIAA (nmoles) 1	5-HIAA (nmoles) 2	Average % inhibition: PAA	Average % inhibition: 5-HIAA
None	2·614	2·407	0·453	0·238		
$2·5 \times 10^{-4}$	0·584	0·370	0·144	0·095	81·1	64·2
$1·0 \times 10^{-4}$	1·004	0·635	0·221	0·147	67·6	44·7
$1·0 \times 10^{-5}$	2·328	1·824	0·407	0·216	17·6	9·7
$1·0 \times 10^{-6}$	2·485	1·973	0·428	0·206	11·4	8·9

* Reaction mixtures containing either 3·6 nmoles PEA or 5-HT, 2·4 mg protein and varying amounts of imipramine, as indicated, in a total volume of 2 ml buffer, were incubated at 37° for 5 min when PEA was substrate and for 15 min when 5-HT was substrate.

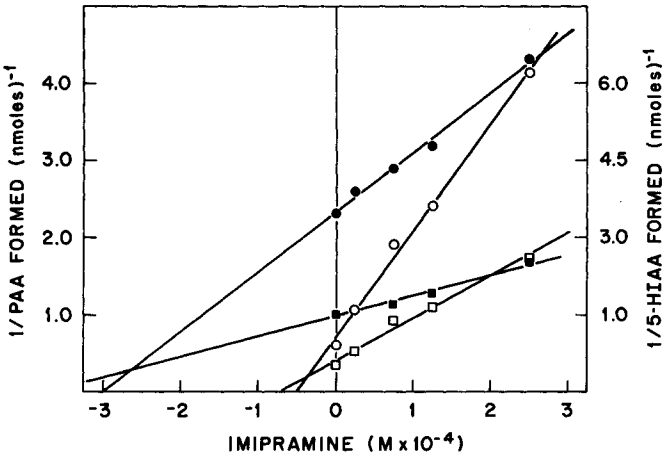

FIG. 3. Dixon plot of deamination of PEA and 5-HT by brain monoamine oxidase (MAO). ^{14}C-PEA, 3·6 μM (–○–○–) and 9·0 μM (–□–□–), was incubated for 5 min at 37° in the presence of varying amounts of imipramine at a protein concentration of 2·6 mg protein/ml of buffer. ^{14}C-5-HT, 0·8 μM (–●–●–) and 1·8 μM (–■–■–), was incubated for 15 min at 37° in the presence of varying amounts of imipramine at a protein concentration of 1·8 mg protein/ml of buffer. Deaminated products formed in the reaction were assayed according to methods described in the text.

The binding constants for both type A and type B brain MAO were determined from the Dixon plots[14] of 5-HT and PEA deamination shown in Fig. 3. The K_i of imipramine for the type A and type B forms of MAO are approximately $2{\cdot}7 \times 10^{-4}$M and $4{\cdot}0 \times 10^{-5}$M respectively. These data are consistent with results reported in Tables 1 and 2 indicating that imipramine has a greater affinity for the B form of the oxidase and thus is expected to be more effective in preventing deamination of PEA.

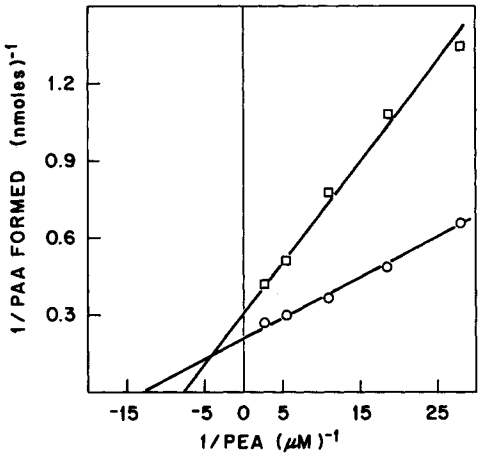

FIG. 4. Lineweaver–Burk plot showing the inhibition of lung monoamine oxidase (MAO) deamination of PEA. ^{14}C-PEA, 1·8 μM, in the absence (–○–○–) and in the presence (–□–□–) of 5×10^{-5}M imipramine, was incubated with lung mitochondria (1·2 mg protein) for 5 min at 37°. Phenylacetic acid and/or aldehyde formed was assayed according to methods described in the text.

TABLE 3. REVERSIBLE NATURE OF IMIPRAMINE BINDING TO LUNG MONOAMINE OXIDASE*

Sample	Amount of product formed (nmoles/mg protein)		% Inhibition	
	PAA	5-HIAA	PAA	5-HIAA
Unwashed				
Control	2·70	0·148		
Imipramine	0·89	0·054	67·1	63·3
Washed				
Control	2·75	0·129		
Imipramine	2·83	0·107	0	16·9

* Experimental details are presented in Methods. Reaction mixtures containing either ^{14}C-phenylethylamine or ^{14}C-5-hydroxytryptamine were done in duplicate. Average values are presented above.

The inhibition by desmethylimipramine of 5-HT deamination has been reported to be of a mixed type.[8] The Lineweaver–Burk plot (Fig. 4) of data from experiments in which lung mitochondrial MAO was used indicates that inhibition of PEA deamination by imipramine is also of a mixed type. Similar results were obtained when mitochondrial MAO preparations from brain were used.

Data presented in Table 3 indicate that repeated washing of mitochondrial preparations restores MAO activity to control values. PEA deamination increased from 0·89 nmole product formed before washing to 2·83 nmoles formed after washing; 5-HT deamination increased from 0·054 nmole to 0·107 nmole 5-hydroxyindole acetic acid (5-HIAA) formed. Thus, since imipramine is removed from mitochondrial preparations by repeated washing, it can be concluded that this antidepressant drug binds reversibly to both the type A and type B forms of MAO.

Since imipramine is extensively degraded to *N*-demethylated products, which also possess antidepressant characteristics,[15] the effect of several *N*-substituted iminodibenzyl analogs on the deamination of PEA was also examined and results are presented in Table 4. The magnitude of inhibition does not appear to be influenced by methyl substituents on the nitrogen of the aliphatic side chain. The primary (didesmethylimipramine), secondary (desmethylimipramine) or tertiary (imipramine)

TABLE 4. INHIBITION OF PEA DEAMINATION BY IMIPRAMINE, DESMETHYLIMIPRAMINE, DIDESMETHYLIMIPRAMINE AND IMIPRAMINE *N*-OXIDE*

Iminodibenzyl analog	Amount of PAA formed (nmoles ± S.D.)	% Inhibition
None	1·922 ± 0·028	
Imipramine	0·674 ± 0·034	64·9
Desmethylimipramine	0·732 ± 0·051	61·4
Didesmethylimipramine	0·632 ± 0·034	67·1
Imipramine *N*-oxide	1·749 ± 0·043	9·0

* Reaction mixtures containing 3·6 nmoles PEA, 0·45 mg brain mitochondrial protein and 0·125 μmole of an iminodibenzyl analog in a total of 2 ml buffer were incubated for 10 min at 37°. All determinations were done in triplicate.

amine, each at a concentration of $6{\cdot}25 \times 10^{-5}$M, inhibits type B MAO by 60–70 per cent. However, the ability of imipramine to inhibit MAO is greatly diminished by addition of an oxygen molecule on the nitrogen, as shown in Table 4 for imipramine *N*-oxide.

DISCUSSION

PEA has been shown by Nakajima *et al.*[16] and Sabelli *et al.*[17] to cross the blood–brain barrier readily. Thus, unlike other biogenic monoamines, it is anticipated that circulating levels of PEA may greatly influence the concentration of this monoamine in brain. Also, drugs which influence blood levels of this substrate (e.g. by inhibition of PEA deamination) would therefore be expected to alter brain PEA levels. Fischer *et al.*[3] previously reported that imipramine increased PEA levels in rat brain and human urine, and results of the present study suggest that this increase may result from imipramine inhibition of PEA deamination in brain, lung and, presumably, also other tissues which contain type B MAO. Imipramine inhibition of both lung and brain MAO appears to be reversible and apparently is unaffected by *N*-demethylation by the mixed-function oxidase enzyme system, since desmethylimipramine and didesmethylimipramine are as effective as the parent drug in inhibiting deamination of PEA. However, the naturally occurring *N*-oxide metabolite of imipramine has little effect on MAO at the concentration used (Table 4).

The lung has been found to accumulate and metabolize 5-HT and NE,[18,19] but little information is available concerning uptake or disposition of PEA in the lung. The rat lung has been shown to contain both PEA[20] ($4{\cdot}0 \pm 1{\cdot}21$ ng/g) and a metabolite of PEA, phenylethanolamine[21] (87 ± 7 ng/g). Whether the PEA present in lung results from *de novo* synthesis or from its extraction from blood has not been determined. It is known, however, that lung contains aromatic amino acid decarboxylase[22] but not phenylalanine hydroxylase.[23] These conditions would permit synthesis of PEA from phenylalanine in pulmonary tissue. Also, preliminary perfusion studies in this laboratory with PEA indicate that this monoamine is taken up by rabbit lung and is extensively and rapidly metabolized to deaminated products. Since the lung also has been shown to accumulate imipramine.[24,25] this antidepressant drug may appreciably increase levels of extracted or endogenous pulmonary PEA. In this regard, it is significant that Nakajima *et al.*[16] have reported that in the absence of the MAO inhibitor, JB-516, essentially no PEA could be detected in several rabbit tissues, including the brain; upon treatment with JB-516, however, PEA was detected in brain and several other tissues. (Lung was not examined.)

In the present study, the concentration of imipramine required to inhibit deamination of PEA by 50 per cent in rabbit lung and brain is approximately 4×10^{-5}M or 11·2 μg/g of tissue. After two intravenous doses of imipramine (each 10 mg/kg) to rabbits, Dingell *et al.*[26] reported that the levels of imipramine in the lung and brain of these animals 1·5 hr after administration were 61·8 and 12·4 μg/g of tissue respectively. Therefore, the concentration of imipramine accumulated in these tissues would be sufficient to inhibit the deamination of PEA by at least 50 per cent. In fact, the concentration of imipramine accumulated in lung is sufficient to inhibit PEA deamination approximately 80 per cent (Fig. 2). Furthermore, the effective imipramine concentration which can inhibit either lung or brain MAO may even be greater, since this drug may be localized in specific areas of these tissues.

The data presented in this paper indicate that imipramine inhibition of mitochondrial MAO could produce the increase in PEA levels observed in rabbit brains after administration of this antidepressant drug and, therefore, may also account for the increase of urinary PEA found in depressed patients treated with imipramine or imipramine-like drugs. Inhibition by imipramine of the type A form of MAO may also account for the decrease in NE deaminated products found in urine and brain of rats receiving acute or chronic doses of imipramine,[27] and likewise may account for the decrease of these NE metabolites in urine of depressed patients receiving this antidepressant agent.[28] The relationship between the ability of imipramine to inhibit both the type A and B forms of mitochondrial MAO and the antidepressant action of this material is not known, but it is tempting to speculate that imipramine inhibition of the oxidative deamination of NE, 5-HT, PEA and other biogenic amines may contribute to the clinical action of this drug.

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