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# STUDIES OF MONOAMINE OXIDASES
## INHIBITION OF BOVINE BRAIN MAO IN INTACT MITOCHONDRIA BY TRICYCLIC ANTIDEPRESSANT DRUGS*

FRANCES M. ACHEE and SABIT GABAY†

Biochemical Research Laboratory, Veterans Administration Medical Center, Brockton, MA 02401, U.S.A.
†Section of Psychiatry and Human Behavior, Brown University, Program in Medicine, Providence, RI, U.S.A.

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**Abstract**—Tricyclic antidepressant drugs (TCA) were found to reversibly inhibit monoamine oxidase (MAO) in intact mitochondria of beef brain cortex. $I_{50}$ values were in the range of $10^{-4}$ to $10^{-3}$ M, using chlorimipramine, amitriptyline, desimipramine, imipramine and doxepin. Unlike TCA inhibition reported for MAO in rabbit tissues, the inhibition observed with beef brain MAO was greater for the A-type enzyme, indicated by serotonin (5-HT) deamination, than for the B-type enzyme, indicated by phenylethylamine (PEA) deamination. Chlorimipramine was the most effective of the five tricyclic antidepressant drugs tested for the inhibition of 5-HT deamination, while amitriptyline was the most effective for inhibiting PEA deamination. Kinetic analyses also revealed marked differences in the interaction of the tricyclics with the A form and the B form of MAO. Inhibition was found to be of a mixed type by reciprocal plots, but Dixon plots indicated that the inhibition was parabolic with 5-HT and either linear or hyperbolic with PEA, depending on the TCA used. Mixed inhibitor studies were also carried out, combining a TCA with a selective (clorgyline or deprenyl) or a non-selective (tranylcypromine) MAO inhibitor. Such combinations did not result in a potentiation of inhibition of either the MAO-A or MAO-B type enzyme activity. The present results indicate that the inhibition of MAO may be of only minor significance in the therapeutic efficacy of TCA in the treatment of depression, especially in combined therapy. However, this conclusion must be tempered by the knowledge that there are marked variations in MAO properties from different enzyme sources, as evidenced by these results.

Monoamine oxidase (MAO, monoamine:$O_2$ oxidoreductase, EC 1.4.3.4) has been found to be inhibited by tricyclic antidepressants (TCA) [1–4]. These drugs are used in the treatment of depression and are regarded generally to be effective because of their ability to block the reuptake of the amine neurotransmitters [5–7]. While the inhibition of MAO by the tricyclic antidepressants is not as potent as that by the more conventional MAO inhibitors (MAOI), it has been speculated that the inhibition of MAO by TCA could contribute in some measure to the therapeutic efficacy of these drugs [3, 4, 8, 9]. Recently, several reports have appeared on the use of combined TCA–MAOI therapy for refractory depressions [10–14] and, in such cases, the possibility also exists that a synergistic effect with regard to MAO inhibition could be significant.

MAO is thought to exist as two functionally distinct enzyme forms, A and B, which differ in their substrate preferences, manifested by the ability of selective inhibitors to block the deamination of one substrate, and not another, at low inhibitor concentrations [15]. In this classification of MAO types [16–18], MAO-A is thought to deaminate serotonin and norepinephrine preferentially and is strongly inhibited by clorgyline and harmaline. It is weakly inhibited by deprenyl and pargyline. On the other hand, MAO-B shows a preference for phenylethylamine and benzylamine and is inhibited more strongly by deprenyl than by clorgyline. Other biogenic amines, such as tyramine (TYR) tryptamine (TRY) and dopamine, are thought to be substrates of both enzymes types, indicated by double-sigmoidal dose–response curves with selective inhibitors. The tricyclic antidepressants have been reported also to be selective for the B-form of MAO in some tissues [3, 19, 20].

In this study, we have characterized the TCA inhibition of MAO in intact mitochondria from beef brain cortex, addressing the questions of the differentiation of MAO forms by TCA inhibition and if the inhibition of MAO could be contributing factor in the efficacy of TCA therapy.

### MATERIALS AND METHODS

*Reagents.* All radiochemicals—([1-$^{14}$C]dopamine hydrobromide (6.28 mCi/m-mole), [1-$^{14}$C]β-phenylethylamine hydrochloride (7 mCi/m-mole), [2-$^{14}$C]tryptamine bisuccinate (47.3 mCi/m-mole), [1-$^{14}$C]tyramine hydrochloride (9.20 mCi/m-mole) and [2-$^{14}$C] serotonin binoxalate (17.2 mCi/m-mole)—and the liquid scintillation chemicals were obtained from the New England Nuclear Corp., Boston, MA. The cation resins, AG50W-X8 (200–400 mesh) and Amberlite CG-50 (100–200 mesh), were obtained from the Bio-Rad Corp., Richmond, CA, and the A. H. Thomas Co., Philadelphia, PA, respectively. The following drugs were kindly supplied through the courtesy of the manufacturers as indicated: clorgyline hydrochloride (M & B 9302), May & Baker, Ltd., London, England; tranylcypromine sulfate, Smith, Kline &

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French Laboratories, Philadelphia, PA; imipramine hydrochloride and chlorimipramine hydrochloride, CIBA-Geigy Corp., Summit, NJ; desipramine hydrochloride, Lakeside Laboratories, Milwaukee, WI; doxepin hydrochloride, Pfizer, Inc., Brooklyn, NY; and amitriptyline hydrochloride, Merck, Sharp & Dohme Research Laboratories, West Point, PA. Deprenyl hydrochloride was a gift from Drs. J. Knoll and K. Magyar, Budapest, Hungary.

*Methods.* Mitochondrial fractions were prepared and MAO activity was assayed as described previously [21, 22]. Radiometric assays were used to measure the deamination of [$^{14}$C]-labeled serotonin (0.5 mM), $\beta$-phenylethylamine (0.5 mM), tyramine (1.0 mM), tryptamine (0.2 mM) and dopamine (0.5 mM). The substrate concentrations used were those determined to be optimal for each substrate under the conditions of assay (0.05 M potassium phosphate buffer, pH 7.4; 20 min, 37°, unless otherwise indicated). Protein concentrations were determined by the method of Lowry *et al.* [23], using bovine serum albumin as the standard.

Inhibition was measured with and without preincubation of the enzyme with inhibitor. For studies with preincubation, the enzyme was preincubated with the inhibitor at 37°, for the desired length of time. Then the reaction was started by the addition of the substrate. In studies without preincubation, the enzyme was added to the reaction mixture containing substrate and inhibitor. The degree of inhibition was determined by comparison to control samples which had been assayed under the same conditions as the inhibited samples.

In the mixed inhibitor studies, the enzyme was preincubated with inhibitor, singly or in combination, for 15–20 min at 37°, pH 7.4, prior to the addition of substrate. When two inhibitors are present at the same time, acting on the same system, the inhibition produced by both inhibitors together is defined by:

$$i_{1,2} = i_1 + i_2 - i_1 i_2$$

where $i_1$ and $i_2$ are the fractional inhibitions produced by each inhibitor separately [24]. Theoretical multiple inhibitions calculated from this relationship of the fractional inhibitions were compared with the results of mixed inhibitions determined experimentally.

## RESULTS

The inhibition of MAO in mitochondria from beef brain cortex by tricyclic antidepressant compounds is indicated by the values listed in Table 1, in approximate order of decreasing effectiveness for both drugs and substrates used. Chlorimipramine and amitriptyline were observed to be the most effective of the drugs tested, and 5-HT and dopamine the substrates most affected.

Unlike the conventional monoamine oxidase inhibitors which are predominatly irreversible inhibitors, the tricyclic antidepressant drugs were found to be reversible inhibitors so that preincubation of enzyme with inhibitor prior to starting the assay with substrate did not affect the inhibition obtained. Reversibility of enzyme inhibition was also confirmed by the Ackermann–Potter method [25] in which enzyme velocity in the presence of inhibitor, plotted against enzyme concentration, resulted in a straight line passing through the origin with a slope less than the line for the control or uninhibited enzyme. This is shown in Fig. 1 for imipramine and the deamination of 5-HT, tyramine and PEA.

*Kinetics of inhibition by tricyclic drugs.* Reciprocal plots of the inhibition of 5-HT and PEA deamination by amitriptyline indicate a mixed inhibition (Figs. 2 and 3), with both $K_m$ and $V_{max}$ being affected. From these plots, $K_i$ values were calculated using the slope of the inhibited reaction equal to $K_m/V_{max}\{1 + [I]/K_i\}$. The $K_i$ values using the two substrates were found not to be the same, being $5.52 \pm 1.66 \times 10^{-5}$ M for 5-HT and $1.60 \pm 0.08 \times 10^{-4}$ M for PEA.

The differences in the effects of tricyclic antidepressant drugs on the deamination of the various MAO substrates were more obvious in Dixon plots of the inhibition data. With PEA, linear plots were obtained from which a $K_i$ of $1.69 \times 10^{-4}$ M for amitriptyline

Table 1. Inhibition of MAO in beef brain mitochondria by tricyclic antidepressants*

| Substrate | | [S] (mM) | Chlorimipramine $I_{50}$ (mM) | Amitriptyline $I_{50}$ (mM) | Desipramine $I_{50}$ (mM) | Imipramine $I_{50}$ (mM) | Doxepin $I_{50}$ (mM) |
|---|---|---|---|---|---|---|---|
| 5-HT | (a) | 0.50 | 0.32 | 0.43 | 0.66 | 0.87 | 1.20 |
| | (b) | 0.10 | 0.15 | 0.17 | 0.22 | 0.32 | 0.35 |
| DA | (a) | 0.50 | 0.48 | 0.46 | 0.47 | 0.85 | 1.10 |
| | (b) | 0.167 | 0.20 | 0.20 | 0.24 | 0.46 | 0.40 |
| TYR | (a) | 1.00 | 0.72 | 0.54 | 3.40 | 2.10 | 2.90 |
| | (b) | 0.0625 | 0.20 | 0.16 | 0.38 | 0.51 | 0.55 |
| TYR | (a) | 0.20 | 1.10 | 0.66 | 2.00 | 2.10 | 2.50 |
| | (b) | 0.01 | 0.16 | 0.23 | 0.36 | 0.65 | 0.68 |
| PEA | (a) | 0.50 | 0.79 | 0.49 | 1.30 | 1.60 | 2.20 |
| | (b) | 0.025 | 0.43 | 0.22 | 0.80 | 0.66 | 0.78 |

* Assays were carried out in 50 mM phosphate buffer, pH 7.4 at 37° using (a) saturating substrate concentrations and (b) $K_m$ substrate concentrations. Reaction was started by the addition of enzyme. Per cent inhibition was determined by comparison of inhibited samples with control samples assayed under identical conditions in the absence of inhibitor. $I_{50}$ values (inhibitor concentration required to inhibit 50 per cent of enzyme activity) were estimated from dose–response plots of per cent inhibition vs p$I$ (negative logarithm of inhibitor concentration).

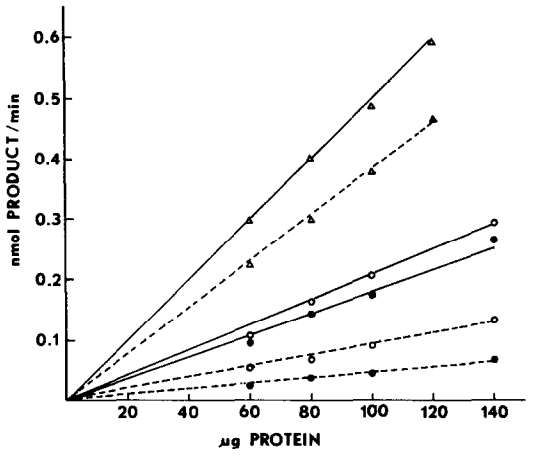


Fig. 1. Effect of protein concentration on MAO inhibition by imipramine. Enzyme activity was determined using $5 \times 10^{-4}$ M [$^{14}$C]-5-HT (●), $5 \times 10^{-4}$ M [$^{14}$C]PEA (○) and $1 \times 10^{-3}$ M [$^{14}$C]TYR (△) in 50 mM phosphate buffer, pH 7.4. Incubations were carried out for 20 min at 37° both in the absence (——) and presence (– – – –) of imipramine. The concentration of imipramine used was $1.25 \times 10^{-3}$ M with 5-HT (●) and PEA (○); $5 \times 10^{-4}$ M imipramine was used to inhibit TYR deamination (△).

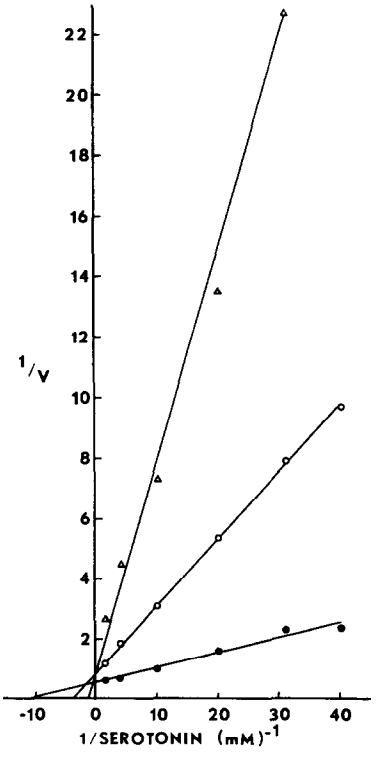


Fig. 2. Lineweaver–Burk plot of amitriptyline inhibition of serotonin deamination. The deamination of varying amounts of [$^{14}$C]5-HT was studied in the absence (●——●) or presence of amitriptyline at $2.5 \times 10^{-4}$ M (○——○) and $5 \times 10^{-4}$ M (△——△) concentrations. Assays were performed in 50 mM phosphate buffer, pH 7.4, incubating for 20 min at 37°, using approximately 100 μg protein/reaction mixture of 0.2 ml; $v$ is in nmoles substrate oxidized/mg of protein/min.

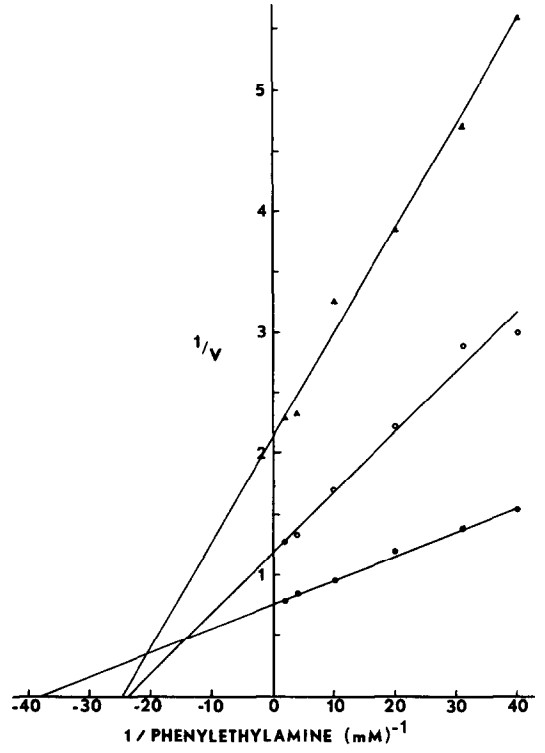


Fig. 3. Lineweaver–Burk plot of amitriptyline inhibition of phenylethylamine deamination. The deamination of varying amounts of [$^{14}$C]PEA was studied in the absence (●——●) or presence of amitriptyline at $2.5 \times 10^{-4}$ M (○——○) and $5 \times 10^{-4}$ M (△——△) concentrations. Assays were performed in 50 mM phosphate buffer, pH 7.4, incubating for 20 min at 37°, using approximately 100 μg protein/reaction mixture of 0.2 ml; $v$ is in nmoles substrate oxidized/mg of protein/min.

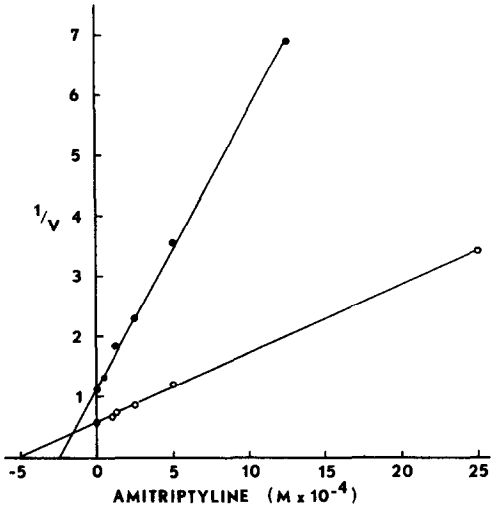


Fig. 4. Dixon plot of amitriptyline inhibition of phenylethylamine deamination. The effects of varying concentrations of amitriptyline on the deamination of $5 \times 10^{-4}$ M [$^{14}$C]PEA (○——○) and $2.5 \times 10^{-5}$ M [$^{14}$C]PEA (●——●) were studied in 50 mM phosphate buffer, pH 7.4, incubating with approximately 100 μg protein/assay mixture of 0.2 ml for 20 min at 37°; $v$ is in nmoles substrate oxidized/mg of protein/min.

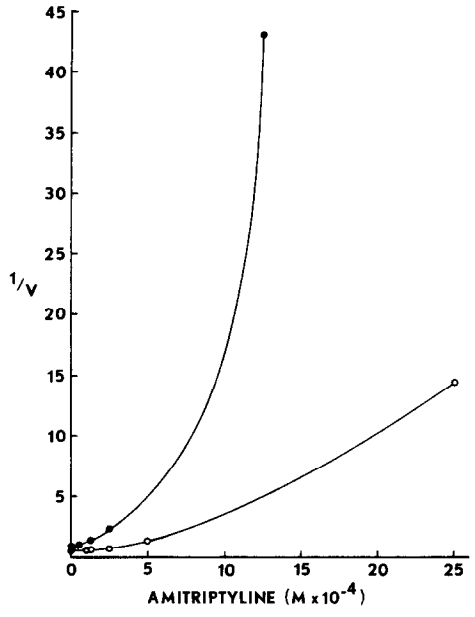


Fig. 5. Dixon plot of amitriptyline inhibition of serotonin deamination. The effects of varying concentrations of amitriptyline on the deamination of $5 \times 10^{-4}$ M [$^{14}$C]PEA (○——○) and $2.5 \times 10^{-5}$ M [$^{14}$C]PEA (●——●) were studied in 50 mM phosphate buffer, pH 7.4, incubating with approximately 100 $\mu$g protein/assay mixture of 0.2 ml for 20 min at 37°; $v$ is in nmoles substrate oxidized/mg of protein/min.

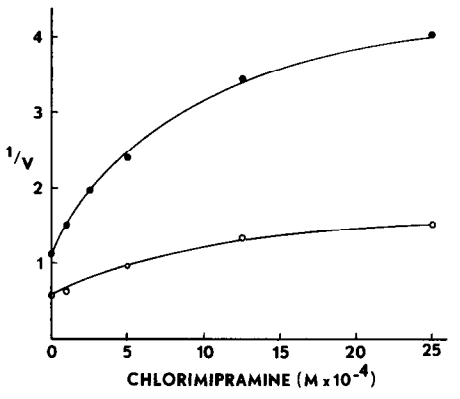


Fig. 6. Dixon plot of chlorimipramine inhibition of phenylethylamine deamination. The effects of varying concentrations of amitriptyline on the deamination of $5 \times 10^{-4}$ M [$^{14}$C]PEA (○——○) and $2.5 \times 10^{-5}$ M [$^{14}$C]PEA (●——●) were studied in 50 mM phosphate buffer, pH 7.4, incubating with approximately 100 $\mu$g protein/assay mixture of 0.2 ml for 20 min at 37°; $v$ is in nmoles substrate oxidized/mg of protein/min.

could be calculated (Fig. 4). The value was in good agreement with the value obtained from the aforementioned reciprocal plots. However, with 5-HT, the Dixon plots were non-linear (Fig. 5), with the curvature indicative of parabolic inhibition. The same type of parabolic inhibition of serotonin deamination was found for all the tricyclics tested. Linear inhibition of PEA deamination was also obtained with imipramine ($K_i = 5.97 \times 10^{-4}$ M), but the inhibition was hyperbolic with chlorimipramine (Fig. 6), desipramine and doxepin. Deamination of tyramine, tryptamine and dopamine also did not display linear inhibition with any of the tricyclics, giving parabolic inhibition, except for desipramine inhibition of tyramine deamination which was hyperbolic.

Table 2. Multiple inhibition of beef brain MAO—Serotonin activity by MAOI and TCA*

| MAO Inhibitor | [MAOI] (M) | Tricyclic Antidepressant | [TCA] (mM) | $i_1$ (MAOI) | $i_2$ (TCA) | $i_1 + i_2$ (Sum) | $i_{1,2}$† (Calculated) | $i_{1,2}$ (Observed) |
|---|---|---|---|---|---|---|---|---|
| Clorgyline | $10^{-6}$ | Imipramine | 0.50 | 89.4 ± 0.9 | 36.0 ± 5.8 | 125.4 | 93.2 ± 0.8 | 90.6 ± 0.4‡ |
| | | | 1.25 | 89.4 ± 0.9 | 81.3 ± 0.9 | 170.7 | 98.0 ± 0.2 | 93.3 ± 1.7‡ |
| | $10^{-7}$ | Amitriptyline | 0.25 | 87.2 ± 1.6 | 35.2 ± 8.1 | 122.4 | 91.4 ± 2.1 | 91.8 ± 0.7 |
| | | | 0.50 | 86.6 ± 1.5 | 68.1 ± 8.4 | 154.7 | 95.5 ± 1.4 | 92.5 ± 1.4 |
| Deprenyl | $10^{-6}$ | Imipramine | 0.50 | 45.0 ± 10.8 | 36.0 ± 5.8 | 81.0 | 64.2 ± 8.8 | 56.5 ± 5.0 |
| | | | 1.25 | 45.0 ± 10.8 | 81.3 ± 0.9 | 126.3 | 98.6 ± 2.5 | 86.1 ± 0.9 |
| | $10^{-7}$ | Amitriptyline | 0.25 | 13.6 ± 3.5 | 35.2 ± 8.1 | 48.8 | 44.4 ± 5.5 | 36.8 ± 6.1 |
| | | | 0.50 | 12.4 ± 5.5 | 58.1 ± 7.2 | 80.5 | 72.1 ± 6.4 | 66.9 ± 3.9 |
| Tranyl-cypromine | $10^{-7}$ | Imipramine | 0.50 | 41.4 ± 6.9 | 38.6 ± 5.4 | 80.0 | 63.8 ± 5.2 | 53.5 ± 5.9 |
| | | | 1.25 | 41.4 ± 6.9 | 82.0 ± 0.1 | 123.4 | 89.6 ± 0.8 | 86.1 ± 1.4§ |
| | $10^{-7}$ | Amitriptyline | 0.25 | 38.0 ± 1.3 | 31.0 ± 5.8 | 69.0 | 57.0 ± 2.4 | 45.8 ± 5.5 |
| | | | 0.50 | 38.0 ± 1.3 | 66.9 ± 4.4 | 104.9 | 79.5 ± 2.3 | 66.5 ± 3.1‡ |

* Inhibition studies were carried out, as described in Materials and Methods, using $5 \times 10^{-4}$ M [$^{14}$C]5-HT as substrate. Values given are mean per cent inhibition ± S.E.M. of three experiments. Per cent inhibition was determined by comparison to control samples containing no inhibitors. Mean control activity: 1.81 ± 0.16 nmoles product/mg of protein/min at 37°.

† $i_{1,2}$ (Calculated) = $(i_1 + i_2) - i_1 i_2$.

‡ $P < 0.025$.

§ $P < 0.05$.

Table 3. Multiple inhibition of beef brain MAO—Phenylethylamine activity by MAOI and TCA*

| MAO inhibitor | [MAOI] (M) | Tricyclic antidepressant | [TCA] (mM) | $i_1$ (MAOI) | $i_2$ (TCA) | $i_1 + i_2$ (Sum) | $i_{1,2}$ (Calculated) | $i_{1,2}$ (Observed) |
|---|---|---|---|---|---|---|---|---|
| Clorgyline | $10^{-6}$ | Imipramine | 0.50 | 60.9 ± 2.4 | 39.7 ± 2.3 | 100.6 | 76.5 ± 1.0 | 68.5 ± 3.6‡ |
| | | | 1.25 | 60.9 ± 2.4 | 70.7 ± 2.4 | 131.6 | 88.7 ± 0.3 | 73.5 ± 3.6§ |
| | $10^{-7}$ | Amitriptyline | 0.25 | 53.1 ± 2.1 | 36.7 ± 1.4 | 89.8 | 70.2 ± 2.0 | 69.4 ± 2.7 |
| | | | 0.50 | 56.5 ± 3.8 | 55.0 ± 6.3 | 111.5 | 80.3 ± 3.0 | 72.8 ± 1.9‡ |
| Deprenyl | $10^{-6}$ | Imipramine | 0.50 | 55.4 ± 11.4 | 39.7 ± 2.3 | 95.1 | 73.2 ± 6.5 | 73.1 ± 4.4 |
| | | | 1.25 | 55.4 ± 11.4 | 70.7 ± 2.4 | 126.1 | 87.3 ± 2.6 | 88.8 ± 3.3 |
| | $10^{-7}$ | Amitriptyline | 0.25 | 47.5 ± 1.1 | 36.7 ± 1.4 | 84.2 | 67.8 ± 1.4 | 69.1 ± 2.1 |
| | | | 0.50 | 45.4 ± 2.7 | 55.6 ± 3.1 | 101.0 | 75.7 ± 2.0 | 75.2 ± 2.9 |
| Tranyl-cypromine | $10^{-7}$ | Imipramine | 0.50 | 52.2 ± 4.9 | 42.0 ± 0.7 | 94.2 | 72.3 ± 2.5 | 66.2 ± 7.4 |
| | | | 1.25 | 52.5 ± 4.9 | 72.4 ± 4.0 | 124.6 | 87.0 ± 0.1 | 80.6 ± 5.1 |
| | $10^{-7}$ | Amitriptyline | 0.25 | 55.1 ± 1.9 | 37.7 ± 0.8 | 92.8 | 72.0 ± 1.5 | 64.2 ± 3.2‡ |
| | | | 0.50 | 55.1 ± 1.9 | 56.9 ± 2.8 | 112.0 | 80.6 ± 2.0 | 72.7 ± 1.7‖ |

* Inhibition studies were carried out, as described in Materials and Methods, using $5 \times 10^{-4}$ M [$^{14}$C]PEA as substrate. Per cent inhibition was determined by comparison to control samples containing no inhibitors. Values given are mean per cent inhibition ± S.E.M. of three experiments. Mean control activity: 1.57 ± 0.12 nmoles product/mg of protein/min at 37°.

† $i_{1,2}$ (Calculated) $= (i_1 + i_2) - i_1 i_2$.

‡ $P < 0.05$.

§ $P < 0.005$.

‖ $P < 0.025$.

*Mixed inhibitor studies.* The combination of clorgyline, deprenyl or tranylcypromine with a tricyclic compound resulted in inhibitions expected on the basis of two inhibitors acting independently on a single enzyme. Results for the effects of mixed inhibitors on the deamination of 5-HT and PEA are given in Tables 2 and 3. Two concentrations of the tricyclic compound were tested with a single concentration of the MAO inhibitor. In general, the experimentally determined inhibitions with mixed inhibitors were not significantly different from the calculated inhibitions. Slightly significant differences were observed for the combination of imipramine and clorgyline inhibiting 5-HT and PEA deamination at both concentrations of the tricyclic compound, and the mixture of imipramine at the higher concentration and tranylcypromine acting on 5-HT deamination. Amitriptyline at both high and low concentrations with tranylcypromine and at the higher concentration with clorgyline also resulted in significant differences between calculated and observed inhibition, with PEA as substrate. In all cases where differences were found, the observe inhibitions were less than those expected from theoretical calculations.

## DISCUSSION

The present results confirm those of other studies that the tricyclic antidepressant drugs do inhibit the action of MAO *in vitro*. However, unlike previous reports, the effects of TCA on beef brain cortex MAO do not appear to be more specific for the B form. Studies on MAO from rabbit tissues and human blood platelets showed that PEA deamination was more affected than the deamination of other substrates, indicated by lower $I_{50}$ and $K_i$ values [3, 4, 8, 19, 20, 26]. As shown in Table 1, with beef brain MAO, there were differences in the $I_{50}$ values among all the substrates and inhibitors tested, but the deamination of serotonin resulted in the lowest values. This indicates that, by the criteria of A and B types of MAO, MAO-A was more affected than MAO-B by the TCA.

This may be a reflection of the differences in enzyme properties often observed with MAO from different species and tissues. In a study of rat muscle MAO, Arora and Meltzer [9] reported that $1.5 \times 10^{-3}$ M imipramine inhibited 5-HT deamination *in vitro* by 77 per cent, but benzylamine deamination by only 57 per cent. The ratio of A to B forms in different tissues has also been reported to vary [27, 28], and in some cases, the general criteria for A and B forms may not always apply. We have shown previously that beef brain MAO differed from MAO in other tissues in that the substrate preferences by the two types were not as sharply defined. Both 5-HT and PEA could be deaminated by either type, as evidenced by double sigmoid-response inhibition curves with clorgyline and deprenyl [29].

Another point of consideration in noting the difference of the effectiveness of the TCA in inhibiting MAO-A vs MAO-B in the system used here, compared to other reports, is the substrate concentration. $I_{50}$ values are influenced profoundly by the substrate concentration [30, 31]. This is seen in Table 1. However, while using $K_m$ concentrations of the substrates resulted in lower $I_{50}$ values, the inhibition of 5-HT deamination was still greater than that of PEA deamination.

Kinetic analyses of the inhibition of MAO by TCA revealed that not only are there perhaps basic differences in the enzyme types, but the tricyclics themselves have differing complex interactions with the enzyme. This may be attributable to the structural differences of the various tricyclic antidepressants. Amitriptyline resulted in mixed inhibition of 5-HT, PEA and TYR, but Dixon plots showed that, whereas the inhibition was linear with PEA, the inhibition was parabolic with 5-HT and TYR. Parabolic inhibition is caused generally by the binding of two or more inhibitor molecules to the enzyme [32]. Thus, the $K_i$ derived for 5-HT deamination from the double-reciprocal plot (Fig. 2) is subject to error and may not correspond to an actual constant. All the tricyclic antidepressants tested gave parabolic inhibition of 5-HT deamination. Imipramine also resulted in linear inhibition of PEA deamination, but the

inhibition with chlorimipramine, doxepin and desipramine was hyperbolic which is indicative of partial inhibition. Of the five tricyclic antidepressants tested here, imipramine and amitriptyline, which caused linear inhibition of PEA deamination, are tertiary amines. Doxepin and chlorimipramine are also tertiary amines, but both have an additional charge group in their ring structure which could confer a difference in their binding properties. Desipramine is a secondary amine.

In a study of some structural requirements for TCA inhibition of rabbit brain MAO, Roth and Gillis [19] noted that drugs having a double bond between the ring moiety and the aliphatic side chain (e.g., amitriptyline) were more effective inhibitors than those with a nitrogen atom in the ring (e.g., imipramine). This is also seen in the present study where amitriptyline is found to be more effective than imipramine. In the inhibition of the deamination of PEA and TYR, amitriptyline is the most effective of the TCA, whereas chlorimipramine is the most effective of the TCA when 5-HT, DA and TYR are the substrates. This again points to probable differences in binding sites on the two enzyme forms.

It has been accepted generally that for clinical use, TCA and MAOI should not be used concurrently because of adverse reactions. However, in recent years, there have been several reports citing the use of such combinations for treatment of intractable depression [10–14]. Several reviews on combined TCA–MAOI therapy have stressed that the potential hazards have been exaggerated and that the regimen may be used under proper guidelines [33, 34]. It has been suggested that a synergistic potential may exist in the combination that is beneficial for refractory cases. In our previous study on the effects of selective inhibitors on MAO, it was noted that enzymatic activity with any substrate could be inhibited completely using low concentrations of an A-type and a B-type inhibitor in combination, whereas very high concentrations were needed for the same result if only a single inhibitor was used [29]. Since the TCA were found to also inhibit MAO with some selectivity, it was felt of interest to study the effect of combining a TCA with an MAOI in the inhibition of MAO. No synergistic effect was noted with any of the combinations tested with regard to increased MAO inhibition, either for the A-type or the B-type enzyme (Tables 2 and 3). In all cases, the observed inhibition by combined inhibitors was equal to or slightly less than the theoretical inhibition expected on the basis of the action of the individual inhibitors. This indicates that MAO inhibition *per se* may not be a significant factor in the therapeutic action of combined MAOI–TCA treatment. The possibility exists that the synergistic effect is upon the inhibition of amine uptake, since it has been reported that the antidepressant efficacy of MAOI appeared to be more closely correlated with their ability to block amine uptake rather than to inhibit MAO [35]. One needs also to consider that the reported increased clinical efficacy of combined therapy is due simply to the summation effects of blocked amine deamination and blocked amine uptake, leading to an overall increased level of amine in the synapse. However, more definitive work needs to be done on these possibilities.

At the present time, it is difficult to draw any strong conclusions on the exact significance of MAO inhibition by TCA to the therapeutic efficacy of these drugs. There have been some studies suggesting that MAO is not inhibited by TCA *in vivo* [36–39], while other investigators have reported finding *in vivo* TCA inhibitors of MAO [9, 40]. It was noted above that the inhibition of MAO is affected by substrate concentration such that, as substrate concentration is lowered, the effectiveness of the inhibitors is increased (Table 1). Since the intracellular concentration of amines may vary, one may not be able to arrive at a true assessment of the effect or lack of effect of TCA on MAO *in vivo*. Nevertheless, it is reasonable to expect from the present study and those of other investigators reporting TCA inhibition of MAO that tricyclic antidepressants could exert multiple actions on the metabolism of amine neurotransmitters. The diverse mechanisms would all then contribute to the total modulatory effect. The TCA inhibition of MAO does point to the marked variations in enzyme characteristics and complexities that one observes with regard to the A and B forms, and further studies may prove significant to a better understanding of the basic nature of the enzyme.

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