Biochemical Pharmacology, Vol. 24, pp. 2039–2046. Pergamon Press, 1975. Printed in Great Britain.

# KINETIC STUDIES OF MEMBRANE-BOUND RAT LIVER MONOAMINE OXIDASE

TIMOTHY J. MANTLE* and KEITH WILSON

Department of Biological Sciences, Hatfield Polytechnic, P.O. Box 109, Hatfield AL10 9AB, England

and

ROBERT F. LONG

Department of Biochemistry, Roche Products Ltd., Broadwater Rd., Welwyn Garden City, England

(*Received* 13 *February* 1975; *accepted* 7 *April* 1975)

**Abstract**—(1) Competition experiments have been carried out with membrane-bound rat liver monoamine oxidase using tyramine, serotonin, dopamine, tryptamine, phenylethylamine, benzylamine and noradrenaline. Simple competitive kinetics were exhibited by most pairs of substrates. (2) Replots of the slopes of Lineweaver–Burk plots against inhibiting substrate concentration were linear in most cases but were hyperbolic for serotonin and α-methyl-tryptamine inhibition of tyramine and dopamine oxidation, and parabolic for noradrenaline and dopamine inhibition of tryptamine oxidation. (3) Simulation using an enzyme model having two independent catalytic sites was only partially successful in producing non-linear replots of the types obtained experimentally. (4) The nature of the catalytic sites of membrane-bound monoamine oxidase is discussed in the light of these results.

There is evidence from inhibitor studies [1–4] thermal stability [5, 6] and pH optima [7, 8] that membrane-bound preparations of monoamine oxidase contain more than one form of the enzyme. Most of the available information is adequately described by the binary enzyme model originally proposed by Johnston [1].

We have recently reported [9] the characterisation of two centres of amine oxidation associated with a membrane-bound preparation of rat liver monoamine oxidase, which differ in their sensitivities towards 5-phenyl-3-(*N*-cyclopropyl)ethylamine-1,2,4-oxadiazole (PCO). One site, designated the A-site, which deaminates serotonin was PCO sensitive, whereas the other, designated the B-site was relatively insensitive to the inhibitor and was responsible for benzylamine oxidation. Tyramine, tryptamine and dopamine were shown to be substrates for both sites. These substrate specificities are in agreement with those originally described for clorgyline [1, 10]. In addition serotonin was shown to bind relatively weakly to the B-site, which was shown to be kinetically similar to the A-site with tyramine, tryptamine and dopamine as substrates [9]. We have referred to the activity of A and B-sites, rather than A and B enzymes as originally suggested [1], since no convincing separation of the two hypothetical enzyme species has been demonstrated. In fact there is increasing evidence that the binary nature of the membrane-bound enzyme may result from the activity of one enzyme in different membrane-environments [11, 12].

In view of the uncertainty concerning the heterogeneity of monoamine oxidase it seemed important to characterise carefully the kinetic properties of the enzyme in the membrane-bound state. For a multi-substrate enzyme, substrate competition offered a useful method to study such properties.

Although isolated reports have appeared in the literature of competition experiments these have generally been limited with respect to the substrates used [13, 14] and have utilised relatively insensitive assay procedures [15]. As such experiments might be expected to provide additional evidence for the postulated A- and B-sites, we have conducted a series of competition experiments with a washed mitochondrial preparation from rat liver, using a sensitive radioassay.

## MATERIALS

[1-$^{14}$C]serotonin creatinine sulphate, [1-$^{14}$C]tyramine hydrochloride, [1-$^{14}$C]dopamine hydrochloride and [G-$^{3}$H]tryptamine hydrochloride were obtained from the Radiochemical Centre, Amersham, Bucks, U.K. Serotonin creatinine sulphate, tyramine hydrochloride, benzylamine and phenylethylamine were obtained from B.D.H. Ltd., Poole, Dorset, U.K. Dopamine, noradrenaline and tryptamine hydrochlorides were obtained from Sigma, London. Anisole was purchased from Koch Light Ltd., Colnbrook, Bucks, U.K. All other reagents were of the highest purity available.

## METHODS

*Preparation of the enzyme.* The enzyme suspension was prepared as previously described [9], and stored in 67 mM-sodium potassium phosphate buffer (pH 7·4) at −15° at a protein concentration of approx 20 mg/ml.

*Determination of enzyme activity.* Monoamine oxidase activity towards tyramine, serotonin, dopamine and tryptamine was determined by the method of

* Present address: Dept of Biochemistry, Tennis Court Rd., Cambridge.

Table 1.

| Substrate | $K_m$ ($\mu$m) | $K_m$ ($\mu$M) data from Weetman and Sweetman [20] |
|---|---|---|
| Tyramine | 155 ± 13 (8) | 45·6 ± 4·6 |
| Dopamine | 256 ± 23 (5) | 171 ± 57 |
| Serotonin | 113 ± 7 (8) | 57·2 ± 9·5 |
| Tryptamine | 12 ± 1·6 (6) | 17·7 ± 7·9 |
| Benzylamine | 106 ± 16 (4) | |

$K_m$ values were obtained from double reciprocal plots covering the substrate concentration range 10 $\mu$M–2 mM for tyramine and serotonin, 5–400 $\mu$M for dopamine and tryptamine and 42 $\mu$M–3·3 mM for benzylamine at 35° and at pH 7·4. The values represent the mean and S.E. of the mean from the indicated number of experiments.

Otsuka and Kobayashi [16] and towards benzylamine by the spectrophotometric method of Tabor *et al.* [17], as described previously [9].

Protein concentration was determined by the method of Lowry *et al.* [18] with bovine serum albumin as standard.

*Treatment of data.* Data obtained from double reciprocal plots in the presence and absence of different levels of inhibiting substrate were analysed by replotting slopes against inhibiting substrate concentrations. Intercepts of double reciprocal plots were not affected by the concentration of inhibiting substrate (i.e. strictly competitive kinetics were observed in all cases). Cleland [19] has shown that for competition at a single site the slope replot is linear, and the intercept on the inhibitor concentration axis is equal to $-K_i$. Following Cleland's nomenclature such plots will be referred to as linear competitive whereas non-linear replots will be called hyperbolic or parabolic respectively for convex or concave upwards curvature. Non-linear slope replots were analysed by extrapolating the initial and final slopes to the inhibitor concentration axis, thus obtaining two apparent $K_i$'s. These will be referred to as $K_{i\text{app}}$ and $K_{ii\text{app}}$, for the apparent high and low affinity modes of binding. For hyperbolic slope replots $K_{i\text{app}}$ is derived at low concentrations of inhibiting substrate and $K_{ii\text{app}}$ at high concentrations of inhibiting substrate. The converse is the case for parabolic replots.

## RESULTS

*Competition experiments.* Linear double-reciprocal plots were obtained with serotonin, tyramine, dopamine, tryptamine and benzylamine as substrates at pH 7·4. The apparent $K_m$ values, shown in Table 1, were consistent from several preparations, and are in agreement with results obtained by Weetman and Sweetman [20] using the oxygen electrode to measure enzyme activity. Reciprocal plots at various levels of inhibiting substrate resulted in strictly competitive kinetics i.e. slope effect; no intercept effect. Most substrate interactions showed linear competitive kinetics (Table 2). However hyperbolic competitive kinetics were exhibited by serotonin inhibition of tyramine (Fig. 1) and dopamine deamination, although serotonin inhibition of tryptamine deamination falls out of an apparent series in exhibiting linear competitive kinetics (Fig. 2). Parabolic competitive kinetics were exhibited by dopamine (Fig. 3) and noradrenaline inhibition of tryptamine deamination. The monoamine oxidase inhibitor α-methyl-tryptamine, which is not a substrate, exhibited hyperbolic competitive kinetics against tyramine deamination (Fig. 4). Also shown in Fig. 4 is the simulated slope replot for inhibition of two independent sites as described below. $K_i$ values for the interactions showing linear competitive kinetics were calculated from slope replots [19] and from Dixon plots [21]. Both methods gave identical results which are shown in Table 3. $K_{i\text{app}}$ and $K_{ii\text{app}}$ derived

Table 2. Inhibition patterns derived from slope replots

| Inhibiting substrate | Substrate: Tyramine | Serotonin | Tryptamine | Dopamine | Benzylamine |
|---|---|---|---|---|---|
| Tyramine | | LC[d] | LC[c] | LC[c] | LC[d] |
| Serotonin | HC[a] | | LC[a] | HC[c] | HC* |
| Tryptamine | LC[d] | LC[d] | | LC[c] | |
| Dopamine | LC[d] | LC[d] | PC[b] | | |
| Benzylamine | LC[d] | LC[d] | LC[b] | | |
| Phenethylamine | C | C | | | |
| Noradrenaline | | LC[d] | PC[b] | | |
| α-methyl-tryptamine | HC[a] | | | | |

Nature of inhibition derived from slope replots of double reciprocal plots. Substrate concentration ranges were 20–100 $\mu$M for tyramine, serotonin and dopamine and 5–20 $\mu$M for tryptamine. The incubations were carried out in the presence of (a) 0, 100, 200, 500, 1000, 2000 $\mu$M; (b) 0, 100, 200, 500, 1000 $\mu$M; (c) 0, 100, 200, 500 $\mu$M; (d) 0, 100, 200 $\mu$M inhibiting substrate at 35° and at pH 7·4. LC: linear competitive; HC: hyperbolic competitive; PC: parabolic competitive; C: competitive, insufficient data for replot; *: data from Sierens and D'Iorio [13].

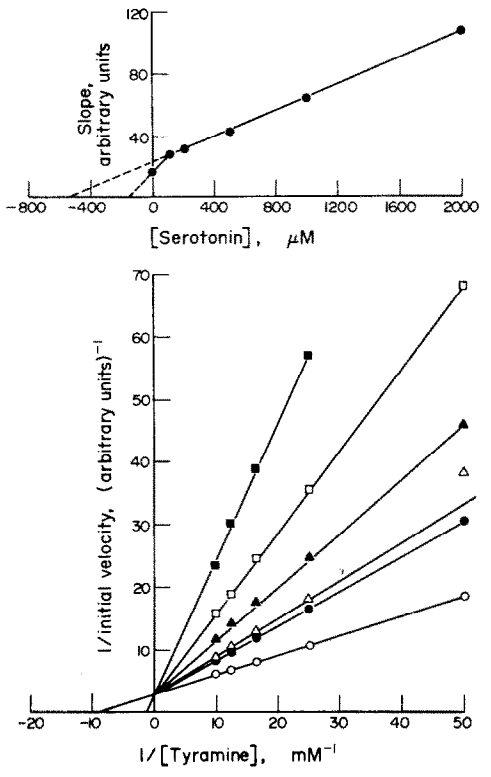



Fig. 1. Double-reciprocal plots of serotonin inhibition of tyramine deamination. The inhibiting serotonin concentrations used were 100 μM (●), 200 μM (△), 500 μM (▲), 1000 μM (□), 2000 μM (■) and zero (○). The slope replot is shown above the reciprocal plot.

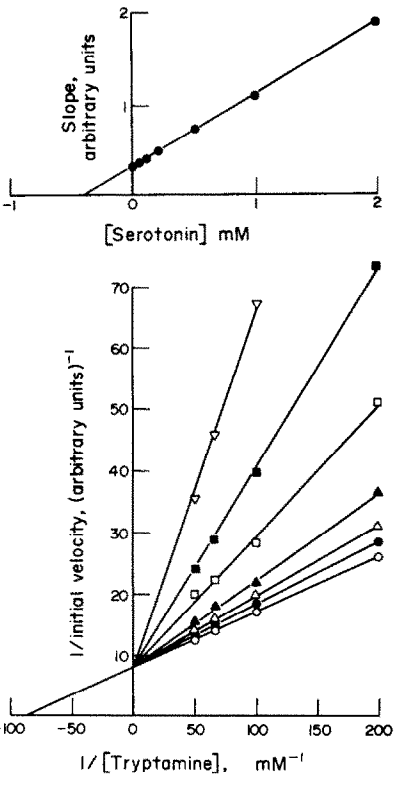



Fig. 2. Double reciprocal plots of serotonin inhibition of tryptamine deamination. The inhibiting concentrations of serotonin were 50 μM (●), 100 μM (△), 200 μM (▲), 500 μM (□), 1000 μM (■), 2000 μM (▽) and zero (○). The slope replot is shown above the reciprocal plot.

from non-linear slope replots as described in the text are shown in Table 4.

*Simulation of the reversible inhibition of two independent sites.* In the light of previous results [9] it was of interest to determine whether the hyperbolic slope replots could result from the inhibition of two independent sites, kinetically equivalent with tyramine and dopamine as substrates, but with different affinities for serotonin. A one-substrate treatment of the competitive inhibition of two independent sites, applicable to the present study as the oxygen concentration was constant, results in the equation;

$$v_0 = \frac{V_1 S}{K_1\left(1 + \frac{I}{K_i}\right) + S} + \frac{V_2 S}{K_2\left(1 + \frac{I}{K_{ii}}\right) + S} \quad (1)$$

where $K_1$, $V_1$ and $K_2$, $V_2$ are the respective $K_m$ and maximum velocities for sites 1 and 2, and $K_i$ and $K_{ii}$ the respective inhibitor dissociation constants for the two sites. While the reciprocal form of this expression is not amenable to analysis for limiting slope functions, it can be simulated manually. The procedure used was to compute $v_0$, with the aid of a digital computer (PDP-10), by inserting values for kinetic constants and substrate and inhibitor concentrations into equation (1). Double reciprocal plots were constructed and analysed by replotting slopes

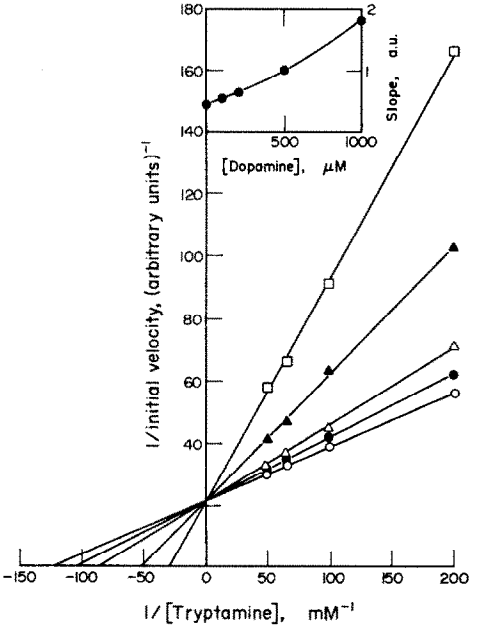



Fig. 3. Double reciprocal plots of dopamine inhibition of tryptamine deamination. The inhibiting concentrations of dopamine were 100 μM (●), 200 μM (△), 500 μM (▲), 1000 μM (□) and zero (○). The slope replot is inset.

Table 3. $K_i$ values ($\mu$M) obtained from the linear competitive data

| Inhibiting substrate | Substrate | | | | |
|---|---|---|---|---|---|
| | Tyramine | Serotonin | Tryptamine | Dopamine | Benzylamine |
| Tyramine | 155 ($K_m$) | 75 | 160 | 160 | 350 |
| Serotonin | | 113 ($K_m$) | 400 | | |
| Tryptamine | 13 | 5 | 12 ($K_m$) | 13 | |
| Dopamine | 185 | 100 | | 256 ($K_m$) | |
| Benzylamine | 100 | 115 | 260 | | 106 ($K_m$) |
| Phenethylamine | 78 | 135 | | | |
| Noradrenaline | | 450 | | | |

against inhibitor concentration. Three general cases were considered:

*Case* 1. When both sites are kinetically equivalent with respect to both the substrate and inhibitor, equation (1) reduces to the linear competitive form:

$$v_0 = \frac{2VS}{K_m\left(1 + \frac{I}{K_i}\right) + S}$$

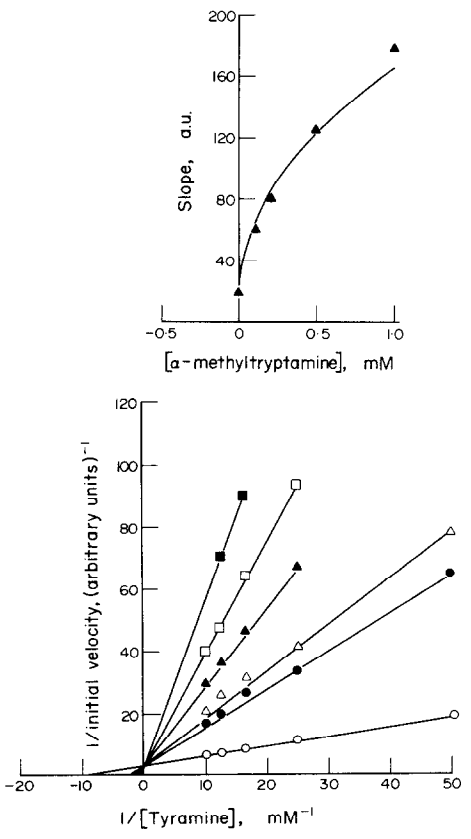



Fig. 4. Double reciprocal plots of α-methyltryptamine inhibition of tyramine deamination. The concentrations of α-methyltryptamine were 100 $\mu$M (●), 200 $\mu$M (△), 500 $\mu$M (▲), 1000 $\mu$M (□), 2000 $\mu$M (■) and zero (○). The slope replot is shown above the reciprocal plot. The parameters used to construct the simulated curve (solid line) according to equation 1 were $V_1 = V_2 = 7{\cdot}05$ a.u.; $K_1 = 130\,\mu$M; $K_2 = 260\,\mu$M; $K_i = 20\,\mu$M; $K_{ii} = 900\,\mu$M. Experimentally derived slope values (▲) are shown for comparison.

*Case* 2. When the two sites are kinetically equivalent with respect to the test substrate (i.e. $K_1 = K_2$; $V_1 = V_2$), but have different affinities for the inhibitor ($K_i < K_{ii}$) simulation revealed hyperbolic competitive kinetics (Figs. 5 and 6). However it can be seen that

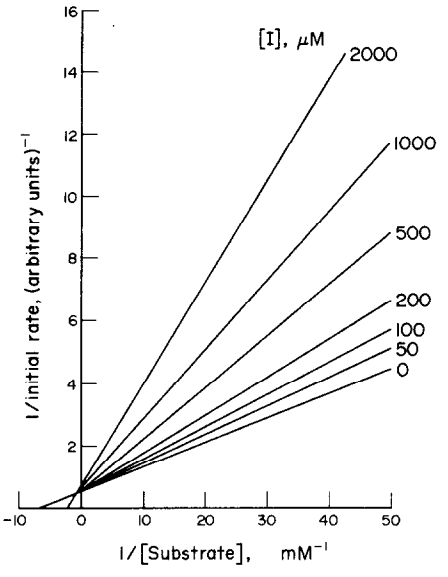



Fig. 5. Reciprocal plots for the simulation of the competitive inhibition of two kinetically equivalent sites (with respect to the test substrate) by an inhibitor with different affinities for the two sites (after equation 1). The parameters used in the simulation were $K_1 = K_2 = 160\,\mu$M; $V_1 = V_2 = 1$; $K_i = 150\,\mu$M and $K_{ii} = 1500\,\mu$M.

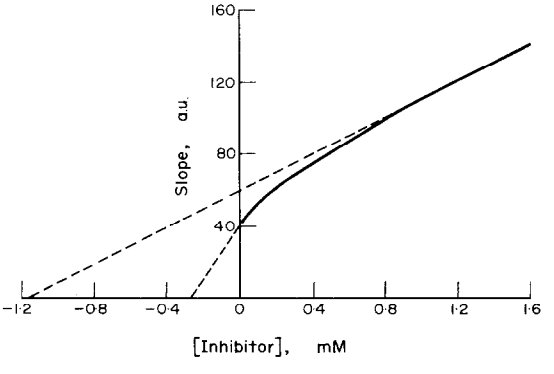



Fig. 6. Slope replot of the data shown in Fig. 5.

Table 4. Apparent $K_i$ values ($\mu$M) derived from the non-linear competitive data

| Substrate | Tyramine | | Dopamine | | Tryptamine | |
|---|---|---|---|---|---|---|
| | $K_{iapp}$ | $K_{iiapp}$ ($\mu$M) | $K_{iapp}$ | $K_{iiapp}$ ($\mu$M) | $K_{iapp}$ | $K_{iiapp}$ ($\mu$M) |
| Inhibiting substrate | | | | | | |
| Serotonin | 130[a] | 520[a] | 160[c] | 800[c] | | |
| Dopamine | | | | | 140[b] | 700[b] |
| Noradrenaline | | | | | 520[b] | 1250[b] |
| α-methyl-tryptamine | 35[a] | 890[a] | | | | |

$K_{iapp}$ and $K_{iiapp}$ derived from extrapolation of the initial and final slopes of slope replots. Substrate concn ranges were 20–100 $\mu$M for tyramine and dopamine and 5–20 $\mu$M for tryptamine. The incubations were carried out in the presence of (a) 0, 100, 200, 500, 1000, 2000 $\mu$M; (b) 0, 100, 200, 500, 1000 $\mu$M; (c) 0, 100, 200, 500 $\mu$M inhibiting substrate at 35° and at pH 7·4.

Table 5. A comparison of real and apparent $K_i$ values derived from simulated slope replots

| Real $K_i$($\mu$M) | | Apparent $K_i$ ($\mu$M) | |
|---|---|---|---|
| $K_i$ | $K_{ii}$ | $K_{iapp}$ | $K_{iiapp}$ |
| 150 | 700 | 240 | 550 |
| 150 | 1500 | 270 | 1200 |
| 150 | 2300 | 275 | 1800 |
| 150 | 10000 | 300 | 5750 |

Apparent $K_i$ values ($K_{iapp}$, $K_{iiapp}$) were obtained by extrapolation of slope replots derived from double reciprocal plots calculated from equation (1), with the real $K_i$ values indicated and $K_1 = K_2 = 150$ $\mu$M; $V_1 = V_2 = 1$. The substrate concentration range was 0·14 $K_1$–0·66 $K_1$, and the inhibitor concentration range was 0·66 $K_i$–13·3 $K_i$.

at inhibitor concentrations above those required to saturate site 1 the strictly competitive nature of the inhibition shifts to a partially mixed competitive type (i.e. a slight intercept effect is observed). This mixed type inhibition was abolished at high substrate concentrations, when the double reciprocal plots became non-linear, curving down towards the $1/v_0$ intercept obtained in the absence of inhibitor. This is not evident in Fig. 5 because the substrate concentration range simulated (20–100 $\mu$M), covers only the linear portion of the curve. The extrapolated lines at substrate concentrations higher than 100 $\mu$M were drawn to indicate the apparent type of inhibition that would be observed under these conditions. Maintaining the same conditions as those simulated in Figs. 5 and 6, the intercept effect becomes more pronounced as the affinity of the second site for the inhibitor is reduced to 10 mM—again saturating with substrate abolishes the intercept effect. Extrapolating the initial and final slopes of the simulated hyperbolic slope replots, derived from the linear portion of double reciprocal plots, to the inhibitor concentration axis (Fig. 6) resulted in two apparent $K_i$ values, $K_{iapp}$ and $K_{iiapp}$ for the apparent high and low affinity modes of binding. These show a reasonable correlation as over and under-estimates of the real values, $K_i$ and $K_{ii}$, providing the inhibitor concentration used is sufficiently high to obtain an accurate estimate of the final slope. This is illustrated in Table 5 where $K_{ii}$ values up to 2 mM are reasonably reproduced by $K_{iiapp}$ values derived from a maximum inhibitor concentration of 2 mM, which concentration is obviously insufficient for a reliable estimate for a $K_{ii}$ or 10 mM.

*Case* 3. When two sites kinetically *similar* with respect to the test substrate, but with different affinities for the inhibitor, are simulated, hyperbolic competitive kinetics are also obtained. Since $K_2$ was found to be greater than $K_1$ experimentally as previously reported [9], simulation was confined to the case where $K_1/K_2 < 1$. If the ratio $K_1/K_2$ was 0·25–0·5, and $V_1/V_2 = 2$, then inhibition by an inhibitor with a $K_i/K_{ii}$ ratio of 0·2–0·5 simulated over the substrate concentration range 0·1 $K_1$–2 $K_1$ resulted in strictly competitive kinetics, although the intercept effect previously noted when $K_1 = K_2$ was apparent at a $K_i/K_{ii}$ ratio of 0·01. The slope replot was hyperbolic over this range. As site 1 was kinetically more significant in this instance the estimates of $K_i$ are better ($K_{iapp}/K_i = 1·2$) than those of $K_{ii}$, where, over a wide range of $K_{ii}$ values a $K_{iiapp}/K_{ii}$ ratio of 2 was obtained. If the $V_1/V_2$ ratio was 1 the intercept effect was not observed at a $K_1/K_2$ ratio of 0·5 and a $K_i/K_{ii}$ ratio

Table 6. A comparison of experimentally determined apparent $K_i$ values ($\mu$M) with apparent $K_i$ values ($\mu$M) derived from simulation studies

| Experimental | | Simulated | | | Simulated | | Experimental | |
|---|---|---|---|---|---|---|---|---|
| $K_{iapp}$ | $K_{iiapp}$ | $K_{iapp}$ | $K_{iiapp}$ | $K_{ii}$ | $K_{iapp}$ | $K_{iiapp}$ | $K_{iapp}$ | $K_{iiapp}$ ($\mu$M) |
| (a) | | | | | | | (b) | |
| 130 | 520 | 190 | 440 | 700 | 210 | 530 | | |
| | | 210 | 650 | 1000 | 220 | 710 | 160 | 800 |
| | | 230 | 1500 | 2300 | 270 | 1630 | | |

Apparent $K_i$ values obtained from hyperbolic slope replots of serotonin inhibition of tyramine (a) and dopamine (b) deamination are taken from the experimental data shown in Table 4. Simulated values were obtained from equation (1) using the data described in the text.

of 0·1. Generally it would appear that in such situations providing neither site becomes kinetically dominant with respect to the test substrate, slope replots can give useful information about the relative $K_i$ values of the two sites, providing sufficiently high concentrations of inhibitor are used to estimate $K_{ii}$.

*Simulation of experimental hyperbolic competitive data.* Apparent $K_i$ values for both sites, derived from experimental slope replots and from simulation according to equation (1) are shown in Table 6. For the simulation the maximum velocities of both sites were taken as equivalent with tyramine and dopamine as substrates [9]. The $K_m$ values for sites 1 and 2, for tyramine and dopamine deamination, were the $K_m$ of the untreated and partially inhibited preparations reported by Mantle *et al.* [9]. It was shown to be unnecessary to correct the $K_m$ observed with untreated preparations for kinetic contributions from the B-site (site 2). The $K_m$ value obtained with serotonin as substrate (120 $\mu$M) was used as $K_i$. Several values of $K_{ii}$ were used in the simulation to find the best fit. From the literature $K_{ii}$ values of 2·4–3·0 mM [9] and 12·5 mM [13] appeared to be candidates but better fits were obtained with a $K_{ii}$ value of approx 1 mM. α-methyltryptamine inhibition of tyramine deamination was simulated using equation (1). Simulated slope values are shown in comparison with those determined experimentally in Fig. 4. All simulations were conducted over substrate and inhibitor concentration ranges used experimentally.

## DISCUSSION

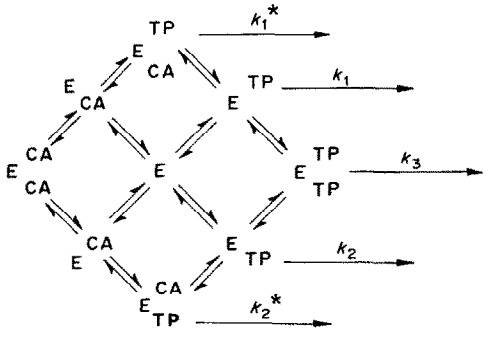
Most of the substrate interactions show linear competitive kinetics with generally good correlation between $K_m$ and $K_i$, as would be expected if all the substrates are binding to a common site, or kinetically indistinguishable sites as described for Case 1. It is interesting to note that one of the apparent $K_i$ values derived from non-linear slope replots also corresponds to the $K_m$ (or $K_i$ against serotonin in the case of noradrenaline) of the inhibiting substrate. Houslay and Tipton [11] have recently reported competitive kinetics for tyramine, tryptamine and dopamine inhibition of benzylamine deamination catalysed by a partially purified enzyme from rat liver. Their $K_i$ and $K_m$ values are equivalent and in good agreement with those reported here.

However the non-linear slope replots suggest that a simple one-site model is too simple to explain the observed results. Since there is a great deal of evidence to suggest that monoamine oxidase is a binary system, three two-site models were considered: (1) two independent catalytic sites, (2) two interacting sites, and (3) two interacting sites only one of which is catalytic. As models 2 and 3 are extremely complex and the resultant rate equations (see e.g. Harper [22]) not amenable to analysis, model 1 was investigated in the form of equation (1) to demonstrate whether it could accommodate all the experimental results, or whether it was necessary to invoke either of the more complex models.

Simulation of the competitive inhibition of two independent sites using equation (1) over the substrate and inhibitor concentrations used experimentally, and with kinetic data derived previously [9], showed that the hyperbolic slope replots could be reproduced using the independent site model. The simulation also raised some interesting points. Thus an examination of the experimental and simulated values of $K_{iapp}$ obtained for serotonin inhibition of tyramine and dopamine deamination (Table 6) indicated that the experimentally determined value was closer to the $K_i$ for serotonin than would be expected from equation (1). Also the simulated slope replots approximated most closely to those obtained experimentally when $K_{ii}$ values of 0·7–1·0 mM were used. These values differ somewhat from those obtained previously. Mantle *et al.* [9] estimated a $K_{ii}$ of 2·4–3·0 mM from kinetic studies with enzyme partially inhibited by an A-site selective inhibitor, whereas Sierens and D'Iorio [13] obtained a value of 12·5 mM from the serotonin inhibition of benzylamine deamination. Sierens and D'Iorio [13] obtained hyperbolic competitive kinetics but did not extrapolate their slope replots. Extrapolation of their data gives a $K_{iapp}$ of 1·5 mM and a $K_{iiapp}$ of 8·5 mM. The result is interesting as the experimentally determined $K_{iiapp}$ values of 520 $\mu$M and 800 $\mu$M obtained for serotonin inhibition of tyramine and dopamine deamination respectively could correspond, as underestimates of the real values, to the $K_{iapp}$ of 1·5 mM obtained by Sierens and D'Iorio (1970) for serotonin inhibition of benzylamine deamination, which, on the independent site model would be an overestimate. The simulated $K_{ii}$ of 1 mM is consistent with such an explanation. This suggestion also receives support from the low affinity site (uncorrected $K_m$ 700 $\mu$M) calculated from the non-linear double reciprocal plot of serotonin oxidation reported by Sierens and D'Iorio [13]. This would also be an underestimate of the real value, if it was the result of serotonin oxidation at two independent sites [23]. The second mode of binding that serotonin inhibition of benzylamine deamination appears to distinguish ($K_{iiapp}$ 8·5 mM) is then possible another example of the apparent binary nature of the clorgyline insensitive activity, as noted by Tipton [24] for the rat liver enzyme using the inhibitor 2-bromo-2-phenylacetaldehyde. It is conceivable that the value for $K_{ii}$ of 2·4 mM obtained in previous work [9] could have resulted from a failure to detect hyperbolic competitive kinetics because too few inhibitory serotonin concentrations were used.

Serotonin inhibition of tryptamine deamination falls out of an apparent series in that it exhibited linear competitive rather than hyperbolic kinetics. This result is unexpected as tryptamine, like tyramine and dopamine is a substrate for both A- and B-sites [10, 9]. The reason for this anomaly is unclear, but may arise from serotonin binding enhancing the rate of oxidation of tryptamine resulting in a parabolic effect on the slope replot as discussed below. As serotonin binds weakly to the B-site, resulting in hyperbolic slope replots, it is possible that a combination of hyperbolic and parabolic slope effects results in a pseudo-linear slope replot. That the linear competitive inhibition of tryptamine deamination by serotonin is anomalous is suggested by the fact that the $K_i$ (400 $\mu$M) is not, as in the other linear competitive cases, a measure of $K_m$ (120 $\mu$M). It is interesting in this respect that it is the inhibition of tryptamine deamination by dopamine and noradrenaline that

resulted in parabolic competitive kinetics. Whereas the hyperbolic competitive kinetics can be partially explained in terms of model (1), although interacting sites could also account for these observations [19], parabolic competitive kinetics cannot result from such a model, and are evidence for an interacting site model. As the parabolic effect was observed only with dopamine and noradrenaline inhibition of tryptamine deamination, and not dopamine inhibition of tyramine or serotonin deamination, or noradrenaline inhibition of serotonin deamination, it is possible that it results from an effect on the catalytic capacity of the enzyme towards tryptamine. Severina and Sheremet'skaya [8] have presented evidence that a 4'-hydroxyl or a 4'-amino group markedly accelerates the rate of deamination relative to the unsubstituted 2-phenyl-ethylamine at pH 7·5 and Houslay and Tipton [25] have concluded that the substrate hydroxyl is important in the formation of a catalytically competent complex at the A-site. It would seem plausible that tryptamine, lacking a hydroxyl substituent, unlike tyramine, dopamine and serotonin, cannot attain the maximum reaction rate. However it is possible that ternary complexes involving catecholamines deaminate tryptamine at an enhanced rate when parabolic kinetics may arise through the following scheme:




where the free enzyme (E) has binding sites A and B($E_B^A$); tryptamine = TP; catecholamine = CA; and where the catalytic constants $k_1$* and $k_2$* are greater than $k_1$ and $k_2$ respectively, which may be greater than $k_3$ on the grounds of reported substrate inhibition with tryptamine. While the effect of depressing the slope value of the double reciprocal plot would occur at low catecholamine concentrations due to the more rapid turnover of the ternary complexes $E_{CA}^{TP}$ and $E_{TP}^{CA}$, at high catecholamine concentrations this effect would be overcome as the ternary complex $E_{CA}^{CA}$ becomes the kinetically most significant enzyme form. At high catecholamine concentrations therefore, the slope replot would curve upwards (parabolic) such that $K_{iiapp}$ approaches the $K_m$ of the catecholamine as experimentally observed. As tyramine did not result in parabolic competitive kinetics, despite its hydroxyl group, it would appear that the more acidic nature of the catecholamine hydroxyl [26] or the presence of a suitable substituent (OH or $NH_2$) in the 3-position are important in the postulated activation. The latter concept receives support from results obtained with the monoamine oxidase inhibitor AB-15 (1-*m*-amino-phenyl-2-cyclopropylamino-ethanol) which is substituted in the 3-position, and which is a potent inhibitor of tyramine, serotonin and dopamine deamination, but which is relatively ineffective against tryptamine deamination [27].

The interaction of two active sites on a single enzyme species can offer explanation for a number of observations which have been made on MAO. Among these are the parabolic kinetics described above, the substrate specific inhibition observed with the compound AB-15 which has already been discussed and the anomalous behaviour towards dopamine oxidation observed with phenylethylhydrazine [28]. On the hypothesis that only two distinguishable active centres exist for the enzyme these effects would be explained by reaction at one site altering the conformation, substrate specificity and catalytic behaviour of the second site.

Alternative explanations exist for all these observations but the existence of A and B sites seems well established by the use of several selective MAO inhibitors. It has proved difficult to separate the hypothetical A and B enzymes physically with full retention of both activities and multiple forms of MAO observed in solubilised preparations cannot be easily fitted in with the properties shown by the selective inhibitors. If the possibility of site interaction is allowed then it seems unnecessary at present to invoke the presence in membrane bound MAO of more than the two sites clearly distinguished by site specific irreversible inhibitors such as clorgyline [1], Deprenil [4] and PCO [9].

## REFERENCES

1. J. P. Johnston, *Biochem. Pharmac.* **17**, 1285 (1968).
2. V. Z. Gorkin and L. V. Tatyanenko, *Life Sci.* **6**, 791 (1967).
3. R. W. Fuller, *Biochem. Pharmac.* **17**, 2097 (1968).
4. J. Knoll and K. Magyar, *Adv. Biochem. Psychopharmac.* **5**, 393 (1972).
5. M. B. H. Youdim and T. L. Sourkes, *Can. J. Biochem.* **43**, 1305 (1965).
6. R. F. Squires, *Biochem. Pharmac.* **17**, 1401 (1968).
7. G. A. Alles and E. V. Heegard, *J. biol. Chem.* **147**, 487 (1943).
8. I. S. Severina and T. N. Sheremet'evskaya, *Biokhimiya* **34**, 125 (1969).
9. T. J. Mantle, K. Wilson and R. F. Long, *Biochem. Pharmac.* **24**, 000 (1975).
10. D. W. R. Hall, B. W. Logan and G. H. Parsons, *Biochem. Pharmac.* **18**, 1447 (1969).
11. M. D. Houslay and K. F. Tipton, *Biochem. J.* **135**, 173 (1973).
12. K. F. Tipton, M. D. Houslay and N. J. Garrett, *Nature, Lond.* **246**, 213 (1973).
13. L. Sierens and A. D'Iorio, *Can. J. Biochem.* **48**, 659 (1970).
14. Y. Kobayashi and R. W. Schayer, *Archs Biochem. Biophys.* **58**, 181 (1955).
15. H. Blaschko, D. Richter and H. Schlossmann, *Biochem. J.* **31**, 2187 (1937).
16. S. Otsuka and Y. Kobayashi, *Biochem. Pharmac.* **13**, 995 (1964).
17. C. W. Tabor, H. Tabor and S. M. Rosenthal, *J. biol. Chem.* **208**, 645 (1954).
18. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
19. W. W. Cleland, in *The Enzymes* (Ed. P. D. Boyer), 3rd Edn. Vol. 2, p. 1, Academic Press, New York (1970).

20. D. F. Weetman and A. J. Sweetman, **41**, 517 (1971).
21. M. Dixon, *Biochem. J.* **55**, 170 (1953).
22. E. T. Harper, *J. theor. Biol.* **39**, 91 (1973).
23. I. M. Klotz and D. L. Hunston, *Biochemistry* **10**, 3065 (1971).
24. K. F. Tipton, *Biochem. Pharmac.* **18**, 2559 (1969).
25. M. D. Houslay and K. F. Tipton, *Biochem. J.* **139**, 645 (1974).
26. I. K. Mushahawar, L. Oliner and A. R. Schulz, *Can. J. Biochem.* **50**, 1035 (1972).
27. Z-S. Huszti, M. Fekete and A. Jahos, *Biochem. Pharmac.* **18**, 2293 (1969).
28. K. F. Tipton, *Adv. Biochem. Psychopharm.* **5**, 11 (1972).