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# β-PHENYLETHYLAMINE AND BENZYLAMINE AS SUBSTRATES FOR HUMAN MONOAMINE OXIDASE A: A SOURCE OF SOME ANOMALIES?

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**Abstract**—Monoamine oxidase (MAO) A predominates both in human placenta and lung. With 5-hydroxytryptamine (5-HT), β-phenylethylamine (PEA) and benzylamine (Bz) as substrates and clorgyline and deprenyl, respectively, as selective MAO A and B inhibitors, their activity pattern has been defined and compared with that of human liver. PEA had a much higher $V_{max}$ with placental MAO A than did Bz; it behaved largely as an A substrate in placenta, and partly as an A substrate in lung. At commonly used substrate concentrations, deamination of Bz (sensitive to $10^{-7}$ M deprenyl) was a better indicator of MAO B activity than deamination of PEA. The divergence between PEA and Bz as MAO A and B substrates may be one reason for some of the apparent discrepancies in the behaviour of MAO A and B noted in a variety of tissues in the literature.

However, Bz reacts with benzylamine oxidase (BzAO) as well as MAO B. Depending on the tissue, deprenyl-resistant Bz activity may indicate the presence of BzAO rather than MAO A. As there is a widespread distribution of BzAO in man and rat, BzAO should be considered among the alternatives of enzyme activity when Bz is used as substrate.

Johnston's classification [1] of monoamine oxidase [MAO; monoamine: oxygen oxidoreductase (deaminating) EC 1.4.3.4] into A and B forms, based on the selective inhibitory action of clorgyline, was to some extent an oversimplification and the premises on which it was founded have latterly come under increasing scrutiny [2–6]. Although, by definition, the classification is based on the action of a particular selective inhibitor, it has often been more convenient to identify the different forms by their preferential action on particular substrates. This practice has brought difficulties in its train. One major problem has been that two substrates commonly assumed (e.g. ref. 7) to distinguish between the two forms, 5-hydroxytryptamine (5-HT) for MAO A, and β-phenylethylamine (PEA) for MAO B, are not completely specific. Both can act as substrates for either form, and any specificity they possess lies in differences in $K_m$ and $V_{max}$ [17, 19]. Moreover, benzylamine (Bz), another substrate for MAO B, is also deaminated by benzylamine oxidase (BzAO) [9, 14]. If one enzyme form is present in great excess, it may contribute the larger part of the activity observed with any of a range of substrates. This could be one explanation of some of the apparent anomalies and differences in the behaviour of MAO A and B recorded for different tissues.

We have recently been engaged in a large-scale investigation of the distribution of BzAO and the two forms of MAO in human tissues [8], using Bz, PEA and 5-HT as substrates. Our criterion for MAO B activity with Bz as substrate is that part of the total suppressed by a deprenyl concentration selective for MAO B, i.e. the deprenyl-sensitive moiety of the activity observed with Bz (D*Bz). The ratio of this moiety to the uninhibited activity registered with PEA as substrate (D*Bz/PEA ratio) tended to be fairly constant and was used by us as a check of the results obtained with either substrate. However, in the course of our study we have come across some notable exceptions. Placenta, lung and some blood vessels have a D*Bz/PEA ratio quite distinct from that observed in the great majority of tissues. We report here the results of a study of MAO A and B in human placenta, lung and liver.

## MATERIALS AND METHODS

Lung and liver samples, obtained at autopsy, were dissected, cleaned and freed from blood by rinsing in cold 0.9% saline, quick-frozen in solid $CO_2$ (−80°), and stored at −20°. The interval between death and autopsy varied between 12 and 24 hr, during which time the bodies were kept at 2–4°. Placentae, obtained from the Labour Ward at Queen Charlotte's Maternity Hospital within 1–6 hr of delivery, were treated similarly. All other procedures involving tissues up to time of assay, and the chemicals used, were as outlined in our previous report [9]. In addition, [$^{14}$C]5-HT, sp.act. 58 mCi/mmole, was purchased from the Radiochemical Centre Ltd., Amersham, U.K., and clorgyline was kindly donated by May & Baker Ltd., Dagenham, U.K.

The basic assay procedure was the radiometric microassay described in detail in our previous paper [9], with the following modifications: the extraction method described for [$^{14}$C]Bz was also used for [$^{14}$C]PEA and [$^{14}$C]5-HT, and the reaction product of the PEA assay was extracted into toluene, whilst that of the 5-HT assay was extracted into a 1:1 mixture of ethyl acetate and toluene.

For the assays shown in Table 1, final concentrations of substrate in the assay mixture were: Bz, 42 μM; PEA, 150 μM; and 5-HT, 371 μM.

Most assays using Bz as substrate were carried out

Table 1. Activity of BzAO, MAO B and MAO A against Bz, PEA and 5-HT. $4 \times 10^{-7}$ M deprenyl was used with Bz to select for MAO B activity. Except where otherwise stated, values represent specific activity (nmoles/mg protein/30 min). For molar concentrations of substrates, see Materials and Methods. Bz assays were carried out at pH 9.0, PEA and 5-HT assays at pH 7.2. Homogenates were 10 per cent (w/v) in 0.1 M potassium phosphate buffer, pH 7.2

| | Bz | | | Ratio | | Ratio | |
|---|---|---|---|---|---|---|---|
| Tissue (N) | BzAO | D*Bz | PEA | D*Bz/PEA | 5-HT | 5-HT/PEA | 5-HT/D*Bz |
| Placenta (4) | 5.2 | 5.1 | 20 | 0.26 | 140 | 7.0 | 27 |
| Lung (3) | 25 | 1.8 | 4.1 | 0.44 | 17 | 4.1 | 9.7 |
| Liver (6) | 6.0 | 80 | 33 | 2.4 | 92 | 2.8 | 1.1 |

with 0.1 M Tris buffer (pH 9.0); this pH has been found by others [10] and by us (unpublished observations) to produce far more activity than pH 7.2, which we had used in our previous experiments [9]. The specific activities recorded in our previous report [9] and those in Table 1 cannot, therefore, be considered directly comparable. As shown by Fowler *et al.* [22], oxidation of tyramine, 5-HT and PEA is inhibited by Tris buffer, but Bz oxidation is not. These findings were confirmed by our experiments with PEA as substrate and Tris buffer (pH 9.0), which produced substantial inhibition (unpublished results). On the other hand, in parallel experiments with potassium phosphate and Tris buffers at pH 7.2 and 9.0, respectively, using the same homogenates, we found that while the higher pH produced far higher activity, the sensitivity of MAO B to deprenyl was the same at pH 7.2 and 9.0 (unpublished results). We therefore considered it justified to use the optimum pH for both Bz (9.0) and PEA (7.2) in these experiments.

The final concentrations of substrate in the assay mixture of the experiments illustrated by Figs. 1 and 2, and Table 2, were as follows: Bz, 42 $\mu$M; PEA, 88 $\mu$M; 5-HT, 217 $\mu$M. To ensure uniformity of results, all these experiments (including those with Bz as substrate) were carried out at pH 7.2 with 0.1 M $K_2HOP_4/KH_2PO_4$ buffer.

Substrate concentrations used in the assays to determine MAO A $V_{max}$ and $K_m$ (Table 3) were as follows: Bz and 5-HT, 500, 250, 125, 62.5, 31.25 $\mu$M; PEA, 250, 125, 62.5, 31.5, 15 $\mu$M.

Several experiments were carried out with a full range of inhibitor concentrations ($4 \times 10^{-3}$ to $4 \times 10^{-9}$ M) and various 10 per cent (w/v) homogenates, the tissue concentration used throughout these experiments. $4 \times 10^{-7}$ M (−)-deprenyl was found to be optimal at distinguishing BzAO from MAO B.

Total tissue protein was determined by the method of Lowry *et al.* [11] with bovine serum albumin as standard.

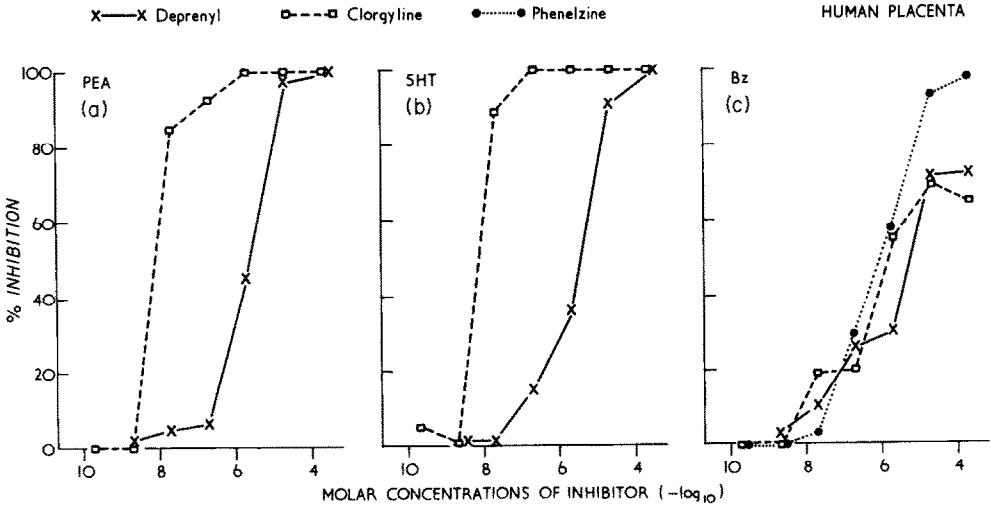


Fig. 1. Effect of different concentrations of clorgyline and (−)-deprenyl on the inhibition of PEA, 5-HT and Bz deamination in human placenta, 10 per cent (w/v) homogenate in 0.1 M potassium phosphate buffer, pH 7.2. All assays carried out at pH 7.2. For substrate concentrations see Materials and Methods. (a) PEA, (b) 5-HT, (c) Bz. □----□ clorgyline, ×——× (−)-deprenyl, ●····● phenelzine. Three separate experiments gave similar curves.

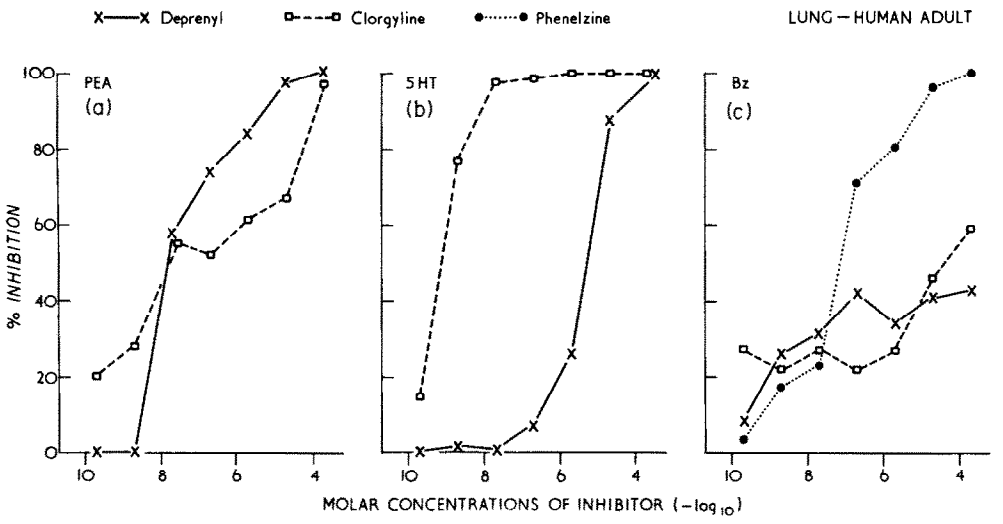


Fig. 2. Effect of different concentrations of clorgyline and (−)-deprenyl on the inhibition of PEA, 5-HT and Bz deamination in human lung. Assay conditions and symbols as in Fig. 1. Three separate experiments gave similar curves.

All assays were carried out in duplicate; values given represent the means of multiple determinations.

## RESULTS

Table 1 shows the specific activities of BzAO, MAO A and MAO B in human placenta, lung and liver, with Bz, 5-HT and PEA as substrates. The D*Bz/PEA ratio, as defined above, is seen to differ substantially in placenta and lung from that observed in liver. In most adult human tissues we have studied [8], the ratio is greater than 2.0, but in lung it is 0.44. The other striking feature of this table is the different pattern of 5-HT/PEA ratios compared with 5-HT/D*Bz ratios.

Figure 1 shows the effect of different concentrations of clorgyline and deprenyl on the inhibition of 5-HT, PEA and Bz deamination in human placenta. It will be seen that 5-HT and PEA gave similar curves (Figs. 1a and b), indicating that a major portion of PEA was metabolized by MAO A. With Bz (Fig. 1c) the pattern was more complicated: a proportion of the activity was not inhibited by either deprenyl or clorgyline and presumably derived from BzAO. This interpretation is borne out by an inhibition curve with phenelzine which, at $4 \times 10^{-5}$ M, blocked 93 per cent of the activity observed with Bz, whereas a tenfold greater concentration of deprenyl and clorgyline achieved only 72 and 65 per cent inhibition, respectively. The remaining activity with these two inhibitors shows a double sigmoid curve, suggesting that some Bz acts as a substrate for MAO A, and some for MAO B.

Figure 2 shows the results of similar experiments on human lung homogenate (10% w/v). With 5-HT as substrate (Fig. 2b), a simple sigmoid curve is seen with both clorgyline and deprenyl, resembling the placental response. The response with Bz and PEA, however, was more complex. Most of the Bz activity was both clorgyline and deprenyl-resistant, but sensitive to $10^{-6}$ M phenelzine, suggesting that it was due to BzAO. The remaining activity was somewhat more sensitive to deprenyl than clorgyline, both inhibitors showing a plateau. With PEA also, a plateau was found with clorgyline, suggesting that the substrate was metabolized by both MAO A and B in this tissue.

When similar experiments were performed with human liver homogenate (10% w/v), 5-HT behaved as a typical MAO A substrate, and Bz and PEA as typical B substrates, all giving single sigmoid plots with both clorgyline and deprenyl.

$I_{50}$ values for the three substrates with deprenyl

Table 2. $PI_{50}$ values for deprenyl and clorgyline inhibition of MAO A and MAO B in human placenta, liver and lung. Values are neg.$\log_{10}$ molar concentrations of inhibitors. For molar concentrations of substrates, see Materials and Methods

| Substrate | Placenta | | Liver | | Lung | |
|---|---|---|---|---|---|---|
| | Dep. | Clorg. | Dep. | Clorg. | Dep. | Clorg. |
| Bz | 5.3 | 5.8 | 8.7 | 5.7 | 3.7* | 4.4 |
| PEA | 5.6 | 8.1 | 7.3 | 4.9 | 7.8 | 7.8 |
| 5-HT | 5.5 | 8.2 | 5.3 | 9.0 | 5.3 | 9.1 |

* Only 42 per cent inhibition was achieved with the highest concentration used, $4 \times 10^{-4}$ M.

Table 3. $K_m$ and $V_{max}$ values for 5-HT, PEA and Bz with placental MAO A, defined as activity sensitive to $10^{-7}$ M clorgyline. These parameters were determined with a mitochondrial preparation from human placenta. $V_{max}$ values are a percentage of MAO activity with 5-HT

| | 5-HT | PEA | Bz |
|---|---|---|---|
| $V_{max}$ | 100 | 13 | 0.7 |
| $K_m$ ($\mu$M) | 200 | 222 | 400 |

and clorgyline against human placenta, lung and liver are shown in Table 2. These values depend on the protein concentration of the homogenate examined.

Table 3 shows the $K_m$ and $V_{max}$ for 5-HT, PEA and Bz with placental MAO A, which is defined as the activity sensitive to $10^{-7}$ M clorgyline. It will be seen that with human placenta, PEA has a much higher $V_{max}$ than Bz and a $K_m$ very close to that of 5-HT; it is therefore a considerably more active substrate of MAO A than Bz.

## DISCUSSION

The striking differences between the D*Bz/PEA ratios of human placenta, lung and liver (Table 1) are at first sight surprising, as both PEA and Bz are generally considered to be substrates for MAO B. Two explanations are possible: (1) MAO B in placenta and adult human lung may differ from that in adult liver and other human tissues; (2) either Bz or PEA, or both, may be acting as substrate(s) for MAO A also and the different contribution of MAO A to total activity in placenta, lung and liver may account for the observed differences in ratios.

The present study shows that it is possible to explain the different D*Bz/PEA ratios (Table 1) without postulating the existence of variant forms of MAO B in the three tissues. In the liver, Bz and PEA are metabolized predominantly by MAO B, and the D*Bz/PEA ratio indicates the relative activity of these two substrates with MAO B. In placenta PEA is deaminated overwhelmingly by MAO A. Relatively little D*Bz activity is seen, both because there is very little MAO B present, compared with MAO A, and because Bz is a much less active substrate for MAO A than PEA (Table 3).

In the lung, the situation is intermediate between placenta and liver, with a significant proportion of activity towards PEA contributed both by MAO A and B. Table 1 shows that in the lung, the A/B ratio expressed by 5-HT/D*Bz is much higher than in the liver, but not so high as in placenta. In the experimental conditions used here, therefore, D*Bz would appear to be a much better measure than PEA of MAO B activity. This conclusion is emphasized by the difference in the A/B ratio expressed by 5-HT/PEA as compared with 5-HT/D*Bz (Table 1).

One of the observations described here has, in fact, been noted previously. The relative activities we observed for 5-HT, PEA and Bz in human placenta are very similar to those found by Egashira [12] who failed, however, to study inhibitor responses with the different substrates. In the human placenta, Bz is oxidized to a slight extent by MAO A (Fig. 1c). Lyles and Callingham [13–15] reported some clorgyline-sensitivity in rat heart, when Bz was employed as substrate, concluding that Bz deamination in that site is achieved, in part, by MAO A. No corresponding activity was detected by Parkinson and Callingham in human heart, however [16]. Working with rat heart, vas deferens and liver, Dial and Clarke [17] claim that PEA acts as a substrate for MAO A, MAO B or a mixture of both, depending on the organ. However, other authors have drawn different conclusions from similar results. For example, Lyles and Callingham find that in rat heart, Bz is a substrate for both MAO A and B, whereas tyramine and PEA are substrates for the A form only; it appears that in this organ, MAO A and B exist in a different form from that of rat liver. Although MAO A or MAO B heterogeneity in a single individual or species cannot be ruled out, the contributions of MAO A, MAO B and BzAO all have to be taken into account before such an inference can be drawn. The biphasic curve found by Lyles and Callingham in rat heart with Bz as substrate [14] resembles that shown here with Bz in human placenta. It seems possible that their results are explicable in terms of different ratios rather than different types of MAO A or B, as rat heart MAO consists predominantly of the A form, whilst liver contains a higher proportion of B. Dial and Clarke's findings with rat vas deferens [17] resemble those reported here with human lung and may have a similar explanation.

The activity obtained with different substrates will depend both on the tissue and on substrate concentrations used. The $K_m$ for PEA with MAO A and MAO B in rat liver has been reported as $6.2 \times 10^{-5}$ M and $4 \times 10^{-6}$ M, respectively [18]. In human platelet MAO B, Edwards and Chang [19] have shown the $V_{max}$ for 5-HT, PEA and Bz to be 7.3, 19 and 41 nmoles, respectively, and the $K_m$, 540, 3 and 130 $\mu$M, respectively. A comparison of these findings with ours (Table 3) for human placental MAO A illuminates our point. Assuming these forms of MAO A and B to be similar to those in other human tissues, we can see that PEA has a much higher affinity for MAO B, so that it will be a more specific substrate at low than at higher concentrations. Bz is more specific than PEA for the B form, in that its $V_{max}$ with MAO B is much higher than that of PEA; conversely, with MAO A, the $V_{max}$ of Bz is much lower than that of PEA. This may account for our results using PEA and Bz at high concentrations in a histochemical study [20], where we found PEA to act as a substrate for both MAO A and B, whereas Bz was specific for MAO B and BzAO.

In defining BzAO and MAO B, respectively, as the deprenyl-resistant and deprenyl-sensitive (D*Bz) moieties of total Bz activity, we are assuming that all deprenyl-resistant activity in the tissues we studied derives from BzAO. A small amount may be due to MAO A, but the present results point to this component being negligible. It is, however, still possible that this deprenyl-resistant BzAO itself consists of more than one enzyme.

Phenelzine is seen to be a potent inhibitor of BzAO (Figs. 1c and 2c). A study of various human tissues at inhibitor concentrations from $4 \times 10^{-3}$ to $4 \times 10^{-10}$ M with Bz as substrate furnished the optimum concentration, $4 \times 10^{-6}$ M, for blocking this enzyme (unpublished results). Unfortunately, in the larger study on human tissues carried out by us (in preparation), it was found that phenelzine is less able than deprenyl or clorgyline to distinguish between the different enzyme forms; at the concentration used, it merely serves to confirm the presence of BzAO, but cannot be considered a selective differentiator by itself.

The data presented here reinforce the view that care must be taken in judging the activities of MAO

A and B in a particular tissue merely from measurements of the oxidation of 5-HT and PEA. At the substrate concentrations generally used, Bz is a more specific substrate for MAO B than PEA, but it also reacts with BzAO. The position can be clarified by using the different substrates with selective concentrations of clorgyline or deprenyl to distinguish the contribution to the total activity of MAO A, MAO B or BzAO.

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*Addendum*—The report of Suzuki *et al.* [23] on the effect of PEA concentration on substrate specificity for MAO A and B, was published after the present paper was written, and is largely confirmed by our findings.

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