Biochem. J. (1988) 256, 911–915 (Printed in Great Britain)

Oxidation of 3-amino-1-phenylprop-1-enes by monoamine oxidase and their use in a continuous assay of the enzyme

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3-Amino-1-phenylprop-1-ene (cinnamylamine) and some derivatives were examined as substrates for monoamine oxidases A and B in mitochondria. All of the amines examined were readily oxidized by monoamine oxidase B but much less readily by monoamine oxidase A. *E*-Cinnamylamine was found to have K_m 0.025 mM and $V_{max.}$ 3.9 nmol/min per mg of mitochondrial protein. Corresponding values with monoamine oxidase A were 0.026 mM and 0.85 nmol/min per mg respectively. Despite their different stereochemistry, *E*- and *Z*-*N*-methylcinnamylamines were almost equally effective as substrates for monoamine oxidase B. The characteristic u.v. absorbance and high absorption coefficient of cinnamaldehyde, the product produced by enzymic oxidation of cinnamylamine, is utilized in a sensitive continuous spectrophotometric assay for both enzymes in the rat and for the assay of a purified monoamine oxidase B from bovine liver.

INTRODUCTION

Amines of the general structure $RCH_2N(R')R''$ usually act as substrates for monoamine oxidase [MAO, amine:oxygen oxidoreductase (deaminating) (flavin-containing), EC 1.4.3.4], particularly when R = aralkyl, R′ = H or Me and R″ = H. One of the reaction products is the aldehyde RCHO. The two known forms of the enzyme, designated A and B, differ in their specificity towards substrates and in their sensitivity to inhibitors. MAO A readily oxidizes 5-hydroxytryptamine, but this amine is a poor substrate for the B enzyme (Tipton *et al.*, 1982). The converse applies to, for example, phenethylamine (Fowler & Tipton, 1982). Some amines, e.g. tyramine, are oxidized well by both enzymes. The $k_{cat.}$ inhibitors *N*-methyl-*N*-propargyl-3-(2′,4′-dichlorophenoxy)propylamine (clorgyline) (Johnston, 1968) and (−)-*N*-methyl-*N*-propargyl-2-phenylisopropylamine [(−)-deprenil] (Knoll & Magyar, 1972) are selective for the A and B enzymes respectively. 3-Amino-1-phenylprop-1-enes (cinnamylamines) have not previously been examined as substrates for the enzyme despite their attractiveness on at least two counts: (i) the existence of *Z*/*E* isomerism suggests that studies of their interaction with MAO might reveal new information about the active site, and (ii) their oxidation should lead to the corresponding cinnamaldehydes, the characteristic electronic spectra of which could be of use in assaying the enzyme. For example, *E*-cinnamaldehyde has a molar absorption coefficient of approx. 24500 $M^{-1} \cdot cm^{-1}$ at 290 nm, whereas the value for *E*-cinnamylamine at this wavelength is about 500 $M^{-1} \cdot cm^{-1}$ (Fig. 1). In this present study the cinnamylamines listed in Table 1 were prepared and examined as substrates for MAO in mitochondria from rat liver. Where necessary, the stereochemistry about the olefinic bond was established by measurement of nuclear Overhauser enhancement (n.O.e.) effects. We show that cinnamylamines are among the most efficient substrates so far reported for MAO B and that K_m and $V_{max.}$ values for the pair of geometrical isomers *E*- and *Z*-*N*-methylcinnamylamines are very similar. We also found that *E*-cinnamylamine, though metabolized less efficiently by MAO A than by MAO B, is nevertheless a useful substrate for this form of the enzyme. It can be used in a sensitive continuous spectrophotometric assay of either or both MAO A and MAO B in whole mitochondria from rat liver, and it is also extremely effective for the assay of MAO B purified from bovine liver. The method compares favourably with other continuous spectrophotometric assays for MAO, such as those described by Tabor *et al.* (1954), with benzylamine as substrate, and by Weissbach *et al.* (1960), in which kynuramine is used, the advantages being due to the high molar absorbance value for cinnamaldehyde and/or to the wavelength (290 nm) at which the enzymic reaction is monitored. The assay is about as sensitive for MAO A as that developed by Houslay & Tipton (1973), in which an excess of a second enzyme, aldehyde dehydrogenase, is used to metabolize the aldehyde produced by the action of MAO on an amine. In this case the reaction rate is measured by the appearance of NADH.

MATERIALS AND METHODS

Chemicals

Kynuramine dihydrobromide was purchased from Sigma Chemical Co., Poole, Dorset, U.K. Clorgyline was synthesized in this laboratory by the method of Bentham *et al.* (1966). (−)-Deprenil was a gift from Professor J. Knoll, Semmelweis University, Budapest, Hungary. Unless otherwise stated all other chemicals were from Aldrich Chemical Co., Gillingham, Dorset, U.K.

MAO

MAO B was isolated from bovine liver by the method of Salach (1979). The enzyme had a specific activity of 32 units/mg of protein. One unit is defined as the amount of enzyme producing an increase in absorbance of 0.0245/min at 290 nm (taken as equivalent to 1 nmol

Abbreviations used: MAO, monoamine oxidase; n.O.e., nuclear Overhauser enhancement.

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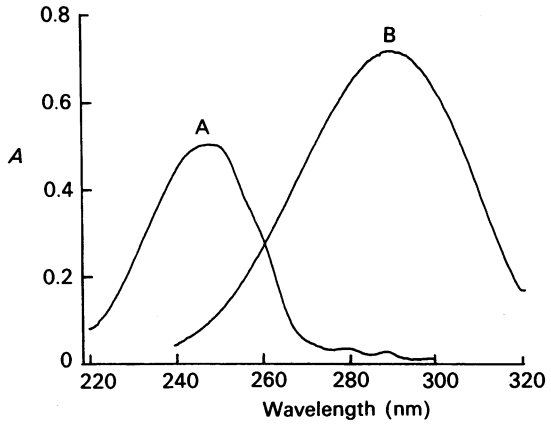

Fig. 1. U.v.-absorption spectra for (A) *E*-cinnamylamine and (B) *E*-cinnamaldehyde, each at a concentration of about 0.03 mM in 0.1 M-phosphate buffer, pH 7.5

Spectra were recorded on a Cary model 118C spectrophotometer.

of *E*-cinnamaldehyde) in a cell of path-length 1 cm at pH 7.5 and 30 °C with *E*-cinnamylamine (0.5 mM) as substrate in a total assay volume of 1 ml. Mitochondria from rat liver were used as a source of MAO A plus MAO B and were isolated and stored as previously described (Williams, 1982). Protein was measured by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Preparation and structure of cinnamylamines

All the compounds were isolated as their hydrochloride salts, which were purified by crystallization, usually from propan-2-ol/diethyl ether mixtures. They were prepared by conventional methods; a brief outline is given for each compound, together with melting point and analytical data for the hydrochloride salts. Compound (I) (Table 1) was prepared by the method of Ishibashi (1965) and was donated by Dr. G. B. Irvine of this Department. Compound (II) was prepared by reduction, with $NaBH_4$ in ethanol, of the Schiff base formed by mixing *E*-cinnamaldehyde and ethanolic methylamine solution (m.p. 148 °C. Found: C, 64.9; H, 7.7; N, 7.9. Calc. for $C_{10}H_{14}ClN$: C, 65.4; H, 7.6; N, 7.6%). The *Z*-isomer (III) was prepared from 3-methylamino-1-phenylprop-1-yne (Simon *et al.*, 1970) by partial catalytic hydrogenation at room temperature and pressure over Lindlar's catalyst with ethanol as solvent. The reaction was stopped when 1 molar equivalent of H_2 had been consumed (m.p. 102–104 °C. Found: C, 65.1; H, 7.7; N, 7.4. Calc. for $C_{10}H_{14}ClN$: C, 65.4; H, 7.6; N, 7.6%). Compound (IV) was obtained from 3-chloro-2-methyl-1-phenylprop-1-ene (Cignarella *et al.*, 1965) by amination by means of the Gabriel synthesis (m.p. 227–228 °C. Found: C, 65.0; H, 7.8; N, 7.6. Calc. for $C_{10}H_{14}ClN$: C, 65.4; H, 7.6; N, 7.6%). Compound (V) was prepared as for compound (II), from α-methyl-*E*-cinnamaldehyde and methylamine (m.p. 171 °C. Found: C, 66.4; H, 8.3; N, 6.9. Calc. for $C_{11}H_{16}ClN$: C, 66.8; H, 8.1; N, 7.1%).

U.v.-absorption spectra of cinnamaldehydes

E-Cinnamaldehyde and α-methyl-*E*-cinnamaldehyde were purified by distillation. *Z*-Cinnamaldehyde was prepared by partial hydrogenation of phenylpropargyl aldehyde diethyl acetal in a manner similar to that described above for the preparation of *N*-methyl-*Z*-cinnamylamine. The resulting *cis*-olefin was treated with acid to release the required aldehyde, which was purified through its bisulphite adduct. The u.v.-absorption spectrum of each aldehyde in 0.1 M-phosphate buffer, pH 7.5, was recorded on a Cary model 118C spectrophotometer. The wavelength maxima ($\lambda_{max.}$) and (in parentheses) molar absorption coefficients were: *E*-cinnamaldehyde, 290 nm (24500 $M^{-1}\cdot cm^{-1}$); α-methyl-*E*-cinnamaldehyde, 290 nm (21500 $M^{-1}\cdot cm^{-1}$); *Z*-cinnamaldehyde, 282 nm (10000 $M^{-1}\cdot cm^{-1}$). The large difference in the ϵ values for the pair of geometrical isomers is in keeping with those for other cinnamyl compounds (see, for example, Grasselli & Ritchey, 1975). The structures of *N*-methyl-*Z*-cinnamylamine, β,*N*-dimethylcinnamylamine and β-methyl cinnamylamine were established by n.O.e. effects in a Bruker WM 250 n.m.r. spectrometer operating at 250 mHz. Spectra were recorded in the difference mode.

Oxidation of cinnamylamines by MAO

Assays were carried out at 30 °C and pH 7.5 in phosphate buffer prepared by mixing 0.1 M solutions of KH_2PO_4 and Na_2HPO_4. Octyl glucoside was then added to a final concentration of 0.1% (w/v). Mitochondria from rat liver were diluted into this buffer to give a protein concentration of 1 mg/ml. Samples (0.95 ml) were placed into each of two cuvettes (path length 10 mm) in a Cary model 118C spectrophotometer. When thermal equilibrium had been attained buffer (0.05 ml) was added to the reference cell and the reaction was started by adding 0.05 ml of substrate solution in buffer to the sample cell. The reaction was monitored by recording the increase in absorbance at 290 nm (282 nm in the case of *N*-methyl-*Z*-cinnamylamine) for periods of up to 3 min. All measurements were carried out in triplicate at eight concentrations of substrate in the range 0.5 mM to 5 μM for each amine. Rates of oxidation were calculated by using the previously determined ϵ values for the aldehydes. K_m and $V_{max.}$ values were obtained by using a computerized weighted least-squares method of linear-regression analysis of $1/[S]$ and $1/v$ values. In all cases the correlation coefficient for the computed slope was 0.97 or greater. To ascertain which form of MAO (A or B) was responsible for the oxidation, these experiments were repeated after first inhibiting one or other enzyme by preincubation for 20 min at 30 °C with either (−)-deprenil (0.5 μM) (to inhibit MAO B) or 0.01 μM-clorgyline (to inhibit MAO A).

Kinetic constants for *E*-cinnamylamine with MAO B from bovine liver were obtained in the same way with 1.75 units of enzyme but with octyl glucoside omitted. For purposes of comparison, K_m and $V_{max.}$ values were measured for oxidation of benzylamine by both rat liver mitochondria and the bovine liver MAO, because this amine is frequently used to assay MAO B in a similar spectrophotometric assay that measures the increase in absorbance at 250 nm due to formation of benzaldehyde (Tabor *et al.*, 1954; see also Tipton & Youdim, 1983). In addition, the oxidation of kynuramine (0.1 mM) by rat liver mitochondria (1 mg of protein) was examined, firstly in the absence of inhibitors and secondly after preincubation of mitochondria for 15 min with (−)-deprenil (1.0 μM) or clorgyline (0.01 μM). This allowed an assessment of the rates of oxidation of kynuramine by MAO A and MAO B under essentially the conditions used by

Weissbach *et al.* (1960), the principal differences being that these earlier workers used a whole-tissue homogenate and carried out their assay at pH 7.4.

A value of 4100 $M^{-1} \cdot cm^{-1}$ was obtained for ϵ_{360} of kynuramine, measured in phosphate buffer at pH 7.5, and this value was used in rate calculations. Weissbach *et al.* (1960) report that a 0.1 mM solution in a cell of path-length 10 mm has an absorbance of 'about 0.5'.

Despite their very different geometry, the kinetic constants for *E*- and *Z*-*N*-methylcinnamylamines with MAO B were remarkably similar (see the Results section). We therefore decided to check that isomerization of *Z*-cinnamaldehyde to the more stable *E*-isomer was not occurring during the enzymic reaction, since this would lead to large errors in calculated velocities. *E*- or *Z*-*N*-Methylcinnamylamine (0.5 mM) was incubated with 0.75 unit of the bovine liver preparation as before, and the spectrum of the reaction mixture was repeatedly scanned over the range 300 nm to 220 nm at intervals of 1 min for a total of 6 min. Thus the rate of decrease in absorbance at 249 nm due to consumption of the *E*-isomer, or at 239 nm due to consumption of the *Z*-isomer, was compared with the increase at 290 nm or at 282 nm due to the formation of an equivalent amount of the corresponding aldehyde.

Use of *E*-cinnamylamine for routine assay of MAO

The K_m value for *E*-cinnamylamine with MAO B was found to be almost identical with that obtained with MAO A (see the Results section), making this amine useful for measurement of total (A + B) activity as well as for assay of the individual enzymes. The assay method is essentially as described above for measurement of K_m values, the substrate concentration being 0.5 mM, which gives a rate of about 95% of $V_{max.}$ for the A and B enzymes in the rat and approx. 87% of $V_{max.}$ for the bovine enzyme. To assess the suitability of the assay over a range of protein concentrations, the enzymic rate was measured with amounts between 0.1 mg and 1 mg of mitochondrial protein and between 22 μg and 110 μg of the partially purified MAO B from bovine liver. In each case the initial concentration of *E*-cinnamylamine was 0.05 mM. The stability of the aldehyde product was assessed by incubating *E*-cinnamaldehyde (10 μM) with the enzyme preparation (1 mg of mitochondrial protein or 55 μg of bovine preparation) for up to 10 min under the conditions of assay. The specificity of the assay was examined by observing the absorbance at 290 nm in the presence of substrate (0.5 mM) after incubating the enzyme preparation for 20 min at 30 °C with the specific $k_{cat.}$ inhibitors clorgyline (0.01 μM) and (−)-deprenil (1.0 μM).

RESULTS

The following n.O.e. data show, for compounds (III), (IV) and (V) (numbering as in Table 1),the irradiated proton, the resonating proton and the percentage enhancement of its resonance: compound (III), Ph–C*H*=C, Ph–C=C*H*, 24.4; compound (III), Ph–C=C*H*, Ph–C*H*=C, 18.5; compound (III), Ph–C=C–CH_2, *ortho-H* of aromatic ring, 26.3; compound (IV), Ph–C*H*=C, Ph–C=C–CH_2N, 20.3; compound (IV), Ph–C*H*=C, Ph–C=C–CH_3, < 1; compound (V), Ph–C*H*=C, Ph–C=C–CH_2N, 19. These data are consistent with an *E*-configuration for compounds (IV) and (V), i.e. the phenyl and aminomethyl groups have a *trans* relationship. The *N*-methylcinnamylamine (III) produced by *cis*-addition of hydrogen to the acetylenic amine clearly has the *Z*-configuration.

Table 1 gives kinetic constants for oxidation of the cinnamylamines by MAO B in mitochondria from rat liver. Data for *E*-cinnamylamine with MAO A of the rat and with MAO B from bovine liver are also given. K_m and $V_{max.}$ values for benzylamine, obtained under the same conditions, were 0.2 mM and 3.7 nmol/min per mg of protein. Corresponding values for benzylamine with the bovine enzyme were 0.194 mM and 41.7 nmol/min per mg. A value of 13800 $M^{-1} \cdot cm^{-1}$ was used for the ϵ_{250} for benzaldehyde (Tipton & Youdim, 1983; von Korff & Wolfe, 1981). Oxidation rates for kynuramine (0.1 mM) under the standard assay conditions, with rat liver mitochondria, were: MAO (A+B), 2.8 nmol/min per mg of protein; MAO A, 0.7 nmol/min per mg; MAO B, 2.2 nmol/min per mg.

For both rat and bovine enzymes the reaction rate with *E*-cinnamylamine was found to be proportional to protein concentration over the range examined. In experiments in which *E*-cinnamaldehyde was incubated with enzyme, the absorbance at 290 nm remained constant, indicating that the product is stable under conditions of assay, though in crude homogenates of tissue the presence of aldehyde dehydrogenase and endogenous NAD^+ might cause some oxidation of the aldehyde to

Table 1. Kinetic constants for oxidation of cinnamylamines, PhCH=C(R)CH_2NHR′, and benzylamine (BZA) by MAO, measured at pH 7.5 and 30 °C

Unless otherwise indicated the data refer to MAO in rat liver mitochondria.

Compound (*E* or *Z*)	R	R′	Enzyme	K_m (mM)	$V_{max.}$ (nmol/min per mg)	$V_{max.}/K_m$ (min^{-1})
(I) (*E*)	H	H	B	0.025	3.14	0.126
(I) (*E*)	H	H	A	0.026	0.85	0.033
(I) (*E*)	H	H	B (bovine)	0.074	34.9	0.472
(II) (*E*)	H	Me	B	0.022	4.1	0.186
(III) (*Z*)	H	Me	B	0.025	3.9	0.156
(IV) (*E*)	Me	H	B	0.017	2.6	0.153
(V) (*E*)	Me	Me	B	0.012	3.1	0.258
BZA			B	0.20	3.7	0.019
BZA			B (bovine)	0.194	41.7	0.215

cinnamic acid, which could affect the behaviour of the assay. The spectral scans made during oxidation of *E*- and *Z*-*N*-methylcinnamylamine by MAO B produced the following results. For the *E*-isomer a new absorbance peak at 289 nm appeared and a decline in the peak at 248 nm was seen. The rate of change at 248 nm was -0.0078 *A* unit/min and that at 289 nm was $+0.0144$ *A* unit/min. These are equivalent to molarity changes for amine and aldehyde respectively of -0.588 μM/min and $+0.594$ μM/min. For the *Z*-isomer the absorbance changes were -0.0036 *A* unit/min at 239 nm and $+0.0035$ *A* unit/min at 280 nm, which gave molarity changes for amine and aldehyde of -0.32 μM/min and $+0.35$ μM/min, assuming an ϵ_{280} value for *Z*-cinnamaldehyde of 10000 $M^{-1} \cdot cm^{-1}$. These results seem to rule out the possibility of any significant isomerization of the *Z*-cinnamaldehyde, for two reasons: firstly, the distinct difference in $\lambda_{max.}$ values for the appearance of products, and, secondly, the substrate/product ratio for the *Z*-isomer is approximately 1 only if the value for *Z*-cinnamaldehyde is used to calculate the rate of product formation.

DISCUSSION

All of the cinnamylamines examined were excellent substrates for MAO B, but were all significantly poorer with MAO A; the maximum turnover rates with the latter were less than 10% of those obtained with MAO B, with the exception of *E*-cinnamylamine itself, its $V_{max.}$ values with MAO A and MAO B being respectively 0.85 and 3.14 nmol/min per mg of protein. Because of their very low rates of oxidation with MAO A K_m values for the other amines with this enzyme were not measured. When enzyme preparations were preincubated with a combination of clorgyline and (−)-deprenil, no change in absorbance occurred at 290 nm when substrate was added, showing that the reaction is specific for MAO. The efficient metabolism of these cinnamylamines by MAO B suggests that the restricted rotation about the double bond presents little problem for the enzyme. This is an interesting contrast with glycine anilide, in which an amide group, with its restricted rotation about the *trans*-C–N bond, replaces the –CH=CH– function of *E*-cinnamylamine. This isostere does not appear to be a substrate for MAO (Williams, 1987). The fact that the β-methyl-*E*-cinnamylamines (IV and V) are oxidized by MAO indicates that the failure of the enzyme to metabolize glycine anilide is unlikely to be due to steric hindrance, and therefore this behaviour may be related more to the polarity than to the size of the carbonyl oxygen atom, which occupies a position spatially equivalent to that of the methyl group in the β-methyl-*E*-cinnamylamines. In addition, the amide group has possibilities for hydrogen-bonding that are absent in the –CH=CH– group. The ability of MAO B to metabolize *E*- and *Z*-*N*-methylcinnamylamines with almost equal efficiency seems remarkable, given their very different spatial characteristics. It has been suggested that, in addition to a binding site for the amino group, MAO has a hydrophobic site that binds the aromatic residue of aralkylamines (Severina, 1973, 1979), a view in keeping with the finding that small aliphatic amines such as methylamine are not substrates (Blaschko, 1963). If such a site exists, than it must be extensive if both *E*- and *Z*-isomers can be equally well accommodated, given that one of the constraints on productive binding of amines is that the enzyme shows absolute optical specificity in removing the pro-*R*-hydrogen from the α-carbon atom (Belleau & Moran, 1963). One explanation is that such a hydrophobic region is part of a very flexible segment of the enzyme that can undergo large changes in conformation.

E-Cinnamylamine is extremly useful for the assay of MAO B. Stock solutions of the amine in buffer are stable for at least 4 weeks at 4 °C. It has a K_m value 10-fold lower than that of benzylamine, and, although its $V_{max.}$ (Table 1) is somewhat lower than that obtained with benzylamine (3.7 nmol/min per mg), this is more than compensated for by the larger value for the molar absorption coefficient of the product, *E*-cinnamaldehyde, compared with that of benzaldehyde. A further advantage is that cinnamylamine oxidation is monitored at 290 nm, whereas the absorbance maximum for benzaldehyde (250 nm) is in a region where large background absorbance can occur, particularly in mitochondrial preparations, which contain relatively large amounts of u.v.-absorbing material other than the enzyme. At a concentration of 0.5 mM, the highest used in these studies, *E*-cinnamylamine has an absorbance of approx. 0.3 at 290 nm. The absorbance spectrum of both substrate and product is unaffected by pH over at least the range 5–9. Another problem that can arise when using particulate preparations such as mitochondria is that their movements in the suspension can lead to irregularities in absorbance, producing erratic recordings [see Tipton & Youdim (1983) for a discussion of the problems associated with the spectrophotometric assay of MAO]. We have found that this problem is eliminated by including the non-ionic detergent octyl glucoside (0.1%). This has the effect of 'dispersing' the mitochondria without affecting either the MAO activity, even when kept at room temperature for 2–3 h, or the absorbance of the assay medium. Experiments with a partially purified MAO B from bovine liver showed that *E*-cinnamylamine is an excellent substrate for assay of this enzyme also. The K_m value is somewhat higher than for the rat liver MAO B (Table 1), but its $V_{max.}$, 37 nmol/min per mg, was comparable with that obtained with benzylamine (41.6 nmol/min per mg). When using this partly purified enzyme it was not necessary to add octyl glucoside to the assay system.

Although rates of oxidation of *E*-cinnamylamine by MAO A were linear for periods of at least 1 h, the rate with MAO B was found to fall off with time. Depending upon the amount of mitochondrial protein used, linearity was maintained for up to 6–7 min. The sensitivity of the assay is such that progress curves of 1–3 min duration were found to be sufficient for calculations of oxidation rates. The reasons for this fall-off in rate are not clear at present, though one possibility is that the reaction product may be capable of inactivating the enzyme by a Michael addition.

Direct spectrophotometric assay of MAO A is used much less frequently than is the case for MAO B, partly because of a dearth of suitable substrates. Weissbach *et al.* (1960) introduced kynuramine as a spectrophotometric substrate for continuous assay of MAO, and Squires (1968) showed that it is oxidized by both forms of the enzyme. The reaction with kynuramine can be followed by monitoring either the fall in absorbance at 360 nm due to consumption of substrate or the increase

in the region 315–330 nm due to formation of 4-hydroxyquinoline, which is produced by a rapid (non-rate-limiting) cyclization of the 2-(*o*-aminobenzoyl)acetaldehyde that is initially formed in the reaction (Weissbach *et al.*, 1960). Houslay & Tipton (1973, 1974) described a coupled assay, suitable for MAO A and MAO B, in which aldehyde dehydrogenase oxidizes the aldehyde produced by the action of MAO on an amine. The reaction is monitored continuously by the increase in absorbance at 340 nm due to formation of NADH. The second enzyme used in this assay is of relatively low specificity, so that it is suitable for use with a wide range of substrates.

The use of *E*-cinnamylamine to assay MAO A seems to offer some advantage over kynuramine. Weissbach *et al.* (1960) reported that kynuramine is oxidized by MAO (A + B, though the existence of the two forms of the enzyme was then unknown) in rat liver at about one-quarter of the rate observed for 5-hydroxytryptamine, since shown to be a substrate predominantly for MAO A (Tipton *et al.*, 1982). This suggested to us that kynuramine is not a particularly good substrate for MAO A in this tissue. However, our results indicate that MAO A contributes about 25% to the total turnover of kynuramine under the conditions used by Weissbach *et al.* (1960), and also show that *E*-cinnamylamine is about 7 times more sensitive than kynuramine for the assay of MAO A. This is mainly a reflection of the higher molar absorption coefficient for the cinnamyl compound.

An advantage over the coupled enzymic assay described by Houslay & Tipton (1973) is that a second enzyme is not required, and, although the sensitivity is limited by the moderate rate of turnover of *E*-cinnamylamine by MAO A (some 3–4-fold less than for a typical substrate such as 5-hydroxytryptamine), the high ϵ value for *E*-cinnamaldehyde compared with that for NADH ($6200\ M^{-1} \cdot cm^{-1}$) means that in practice there is little difference in sensitivity between the two methods. It should be realized, though, that the principal advantage of the coupled assay lies not in its use as a routine procedure but as a way of monitoring by spectral means the oxidation by MAO of amines that are otherwise unsuitable for investigation by spectrophotometric techniques.

Thus *E*-cinnamylamine is a useful addition to the substrates available for the continuous assay of MAO A or B and offers advantages in either convenience or sensitivity over some other spectrophotometric substrates currently in use.

Note added in proof (received 29 September 1988)

Since we submitted this paper, Hiebert & Silverman (1988) have reported that cinnamylamine is a good substrate for MAO but that it has no inactivating effect, even after a 4 h incubation with enzyme. We have now established that our observed fall off in linearity of the progress curve with this substrate is not due to irreversible inhibition (i.e. Michael addition is not occurring) but the enzyme is reversibly inhibited by the product, cinnamaldehyde.

Received 11 April 1988/3 June 1988; accepted 8 June 1988

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