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Oxidation and Enzyme-activated Irreversible Inhibition of Rat and Ox Liver Mitochondrial Monoamine Oxidase-B by 2-(Benzylamino) Acetamide

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Abstract

The interactions of the anticonvulsant 2-n-pentylaminoacetamideanalogue, 2-benzylamino acetamide (FCE 25692) with MAO-B from rat and Ox liver mitochondria have been studied. This compound involves retention of the aminoacetamide portion of the parent compound but replacement of the pentyl moiety with benzylamine which is a good substrate for MAO-B. The results indicated FCE 25692 to be a good substrate for MAO-B from both preparations used with apparent K_m values of 229.8 and 920.0 μM and V_{max} values of 0.230 and 0.989nMol.min^{-1}.mg^{-1} for rat and ox liver mitochondrial MAO-B, respectively. It also acts as a suicide substrate and is a better substrate than it is an inhibitor for MAO-B from both species, with partitions ratios of 816.7 and 2120 mol of product per mol of enzyme inactivated, respectively for rat and ox liver mitochondrial MAOB. The partition ratio for ox liver MAO-B was considerably higher than that of the enzyme from rat liver and the half-life ($t_{1/2}$) of ox liver MAOB was a little larger than its respective ($t_{1/2}$) value for the enzyme from rat liver. The turnover numbers (k_{cat}) and the k_{cat}/K_m values are compared with the inhibition specificity constants (K_{in} /K') these values confirmed the fact that FCE 25692 is a better substrate for rat liver MAO-B than for ox liver MAO-B with kcat/Km values of (118 and 46.1 min^{-1}.mM^{-1}, respectively). While the inactivation constant k_{in} values showed that FCE 25692 is somewhat a better inhibitor for ox liver MAO-B than rat liver MAO-B (0.020 and 0.033, respectively). However the progress curves for the inhibition of MAO-B from both preparations showed that FCE 25692 was a better inhibitor of MAOB from rat preparation than ox preparation.

Keywords: Monoamine Oxidase-B (MAO-B), 2-(benzylamino) acetamide [FCE 25692], Suicide Substrate, Kinetic Parameters.

1. Introduction

Monoamine oxidase (MAO) (EC 1.4.3.4.) is a Flavin-Adenosine-Dinucleotide (FAD)-containing enzyme, (Tipton et al., 2004), associated with the outer membrane of the mitochondriain all mammalian cell types, with the notable exception of the erythrocyte. It converts biogenic amines to their corresponding aldehydes by oxidative deamination according to the following reaction:

$$RCH_2NH + O_2 + H_2O \rightarrow RCHO + NH_3 + H_2O_2$$

Its primary role lies in the metabolism of amines and in the regulation of neurotransmitter levels and intracellular amine stores. Because of the vital role that MAOs play in the inactivation of neurotransmitters, MAO dysfunction is thought to be responsible for a number of psychiatric and neurological disorders for example, unusually high or low levels of MAOs in the body have been associated with schizophrenia (Domino *et al*., 1976*;* Schildkraut *et al.,* 1976) and depression (Meyer *et al*., 2006).

In humans there are two types of Monoamine oxidase, MAO-A and MAO-B. MAO-A is found in both dopaminergic, as well as noradrenergic neurons (Shih *et al*., 2004) and may help eliminate 5-HT in these neurons. Similarly, MAO-B found in serotonergic neurons may protect these cells from the entry of dopamine and2-phenylethylamine (PEA), for which MAO-B shows a preferred affinity (Tipton *et al*., 1975). MAO's role in limiting the presence of these neurotransmitters following their release may secondarily result in altering the release of hormones from cells sensitive to biogenic amines. Thus, MAO may function indirectly as a regulator of neuroendocrine function.

Monoamine oxidase is a well-known enzyme in pharmacology, since it is the substrate for the action of a number of monoamine oxidase inhibitor drugs. In fact, MAO-A inhibitors

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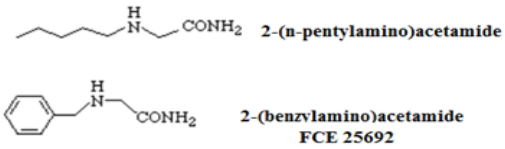
act as antidepressant and antianxiety agents, whereas MAO-B inhibitors are used alone or in combination to treat Alzheimer's and Parkinson's diseases (Riederer *et al.*, 2004), although they are often last-line treatment due to risk of the drug's interaction with diet or other drugs.

The discovery that the antidepressant drug iproniazid (1-isonicotinoyl-2-isopropylhydrazine) was an inhibitor of monoamine oxidase and it belongs to hydrazines, the first known suicide (mechanism-based) enzyme inhibitors of MAO that act as enzyme activated irreversible inhibitors where the active inhibitory species is formed through the action of MAO itself, stimulated the development of many other inhibitors of this enzyme, which have been used as antidepressants, for review see (Tipton, 1989; Ben Ramadan *et al*., 2007; Shulman *et al*., 2013). The benzylamine derivative 2-benzylamino acetamide, also designated as:



FCE 25692, belongs to a series of analogues of milacemide (2-n-pentylamino acetamide) that acts as both a 'suicide' substrate and specific MAO-B inhibitor (Dostert *et al*., 1990), a glycine derivative with atypicalanti-epileptic and potential psychotropic properties acts as a pro-drug delivering glycine into the central nervous system. Janssens de Varebeke *et al*. (1988 & 1989) first suggested that the capability of brain MAO-B to metabolize milacemide to glycinamide with a concomitant increase of brain glycine may be a prerequisite for milacemide'santiconvulsant activity, but it now seems apparent that the 'delivery of glycine to the brain' hypothesis for anticonvulsant activity remains an imperfect model. Despite the doubt on the role of glycine formation in the anticonvulsant actions of milacemide, the possibility of using derivatives of this compound for the delivery of pharmacologically-activecompounds to the brain remains. In the present work comparative kinetic studies on the behavior of 2-benzylaminoacetamideas a substrate and an inhibitor of MAO-B from rat and ox liver mitochondrial preparations have been done. FCE 25692 was reported by Dostert *et al*. (1991), to be a potent and selective rat liver MAO-B inhibitor (IC_{50} = 60 nM, without enzyme-inhibitor preincubation) and to be deaminated at a slow rate as compared with milacemide and some of the other analogues. Furthermore, it displayed little protection against tonic convulsions and death in mice.

2. Experimental Procedures

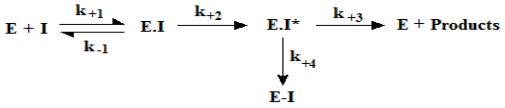
2.1. Materials

Benzylamine HCL was obtained from Sigma Co. (2-(benzylamino) acetamide) FCE 25692 was synthesized at Farmitalia Carlo Erba, Milan, Italy. All other chemicals were standard laboratory chemicals and were of analytical reagent grade whenever possible.

2.2. Methods

Rat liver mitochondria were prepared by the method of Kearney et al. (1971). The mitochondrial pellet obtained was suspended in a small volume of 0.1M potassium phosphate buffer, pH 7.2 and stored at -20oC until use for MAO-B. Ox liver mitochondria were prepared by the method of Salach (1979). All enzyme assays were performed at 37oC and pH 7.2. MAO-B activity was determined spectrophotometrically by directly monitoring the formation of benzaldehyde from benzylamine by following the increase in absorbance at 250 nm Tabor et al. (1954). The reaction mixture contained 93 mM potassium phosphate buffer, pH 7.2, enzyme preparation at the indicated concentrations and (333 µM) benzylamine. The molar extinction coefficient ( ) of benzaldehyde at 250 nm was taken to be 13.8 x 103 M-1.cm-1 (Tipton & Youdim, 1983). The oxidation of the suicide substrate FCE 25692 by MAO was examined by the direct spectrophotometric assay for benzaldehyde formation at 250 nm. The Kinetic constants Vmaxand Km were determined using the computer program ENZFITTER. The double reciprocal plots are used only for illustrative purposes. The reaction progress curves were analyzed using the computer program MACCURVE-FIT to estimate the values of the maximum product formation at time = ∞ (Amax) and the apparent first-order rate constant for the decline in activity with time (kapp).

The partition ratio (r), which represents the number of mol of product formed per mol of enzyme inhibited (or k3/k4)was calculated by determining the amount of product formed at complete inactivation [P∞] for different enzyme inhibitor ratios, according to the relationship (Waley, 1980) $r = (k+3 / k+4) = [P_\infty] / e_0$



(I) FCE 25692, (E) the Enzyme, (E.I) a non-covalent compound, (EI*) an activated intermediate, (E-I) the irreversibly inhibited species

The direct spectrophotometric assay for aldehyde formation was used to examine the reactions of MAO-B (at different concentrations) with FCE 25692 (at different concentrations) while enzyme: FCE 25692 ratios were kept constant. An Uvikon-931 double beam spectrophotometer equipped with a multicellauto changer, which allow the sequential determination of six samples with the appropriate blanks where the temperature was controlled by the use of a circulating water bath was used. The curves were analyzed by the procedure of Waley (1980 & 1985), using the MACCURVE-FIT

computer program. Then the apparent Km value (K') for the inhibition reaction and the inactivation constant (kin) for the inhibition process were determined according to the following relationships, Waley (1980 &1985):

$$I_o t_{1/2} = \left(\frac{\ln (2-M)}{(1-M)}\right) \frac{K'}{k_{in}} + \frac{\ln 2}{k_{in}} I_o$$

$$k_{in} = \frac{k_{+2}\ k_{+4}}{k_{+2} + k_{+3} + k_{+4}} = \frac{\ln 2}{\text{slope}}$$

$$K' = (\text{ordinate intercept}).k_{in} \frac{(1-M)}{\ln(2-M)}$$

where: $M = (1+r).e_o / I_o$

I_o and e_o are the initial inhibitor and enzyme concentration, respectively.

3. Results

3.1. Time-Courses of Oxidation of FCE 25692 by Liver Mitochondrial MAO-B from Both Species

The initial rates of FCE 25692 oxidation, were found to be linear function of the enzyme concentration. The time-courses for the oxidation of FCE 25692 by MAO-B from both species used deviated from linearity after few minutes of starting the reactions. After the reaction had ceased almost completely (55 and 100 min) for rat and ox liver MAO-B, respectively, it could not be restored by the addition of more substrate indicating that the reaction had not ceased due to substrate depletion or the establishment of an equilibrium of a reversible reaction. Neither could any activity be detected when (333µM) benzylamine was added. However, the addition of more enzymes was found to restore the activity as shown in Fig. 1 (it is taken as a representative for the enzyme from both preparations).

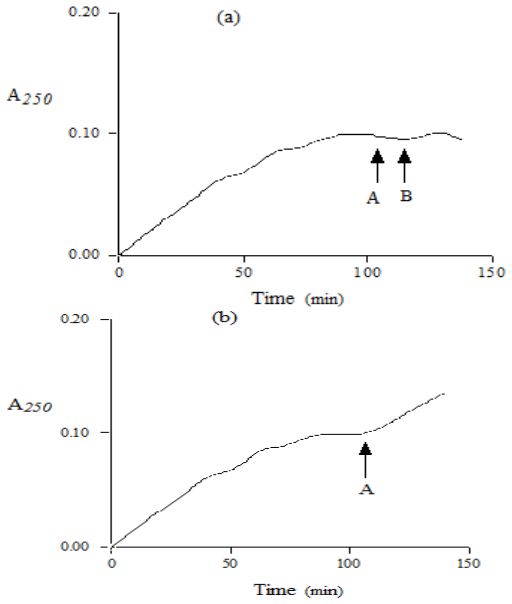


Figure 1. Time Course of Oxidation of FCE 25692 by Rat Liver Mitochondrial Monoamine Oxidase-B

Figs. 2 and 3 show series reaction progress curves of rat and ox liver mitochondrial MAO-B with different concentrations of FCE 25692, respectively. These reactions obeyed the Michaelis-Menten kinetics. The Michaelis constants (Km) and the maximum velocities (Vmax) for the oxidation of FCE 25692 by MAO-B from the enzyme preparations used were determined and compared with their respective values for the parent amine benzylamine. The values obtained are shown in (Table 1).

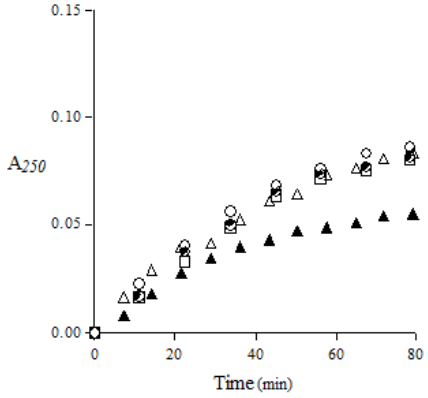


Figure 2. Time Courses of Oxidation of FCE 25692 at a Series of Different Concentrations by Rat Liver Mitochondrial MAO-B.The reactions between rat liver mitochondria (600 µg) and FCE 25692, (▲) 1, (△) 2, (□) 3, (◕) 4, and (⬭)5mM, were followed spectrophotometrically at 250 nm. using the direct assay. The points shown are the results from five representative experiments.

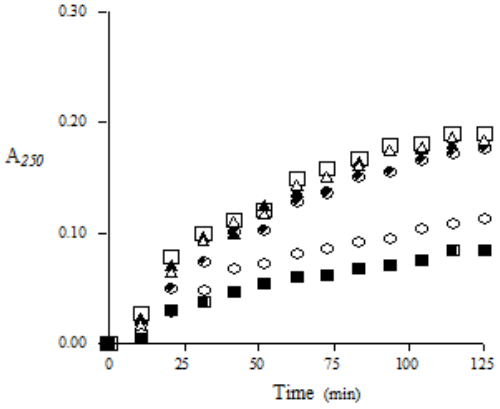


Figure 3. Time Courses of Oxidation of FCE 25692 at a Series of Different Concentrations by Ox Liver Mitochondrial MAO-B.

Table 1. The Kinetic Parameters for the Oxidation of FCE 25692 and Benzylamine by Monoamine Oxidase-B from the two Preparations

Enzyme preparation	Rat Liver Mitochondria	Ox Liver Mitochondria
Substrate	**Benzylamine FCE 25692A**	**Benzylamine FCE 25692A**
$\mathbf{K_m}$ (μM) (2)	211.8 ± 33.9 (3) 229.8 ± 10.0 (3)	144.4 ± 9.8 (2) 920.0 ± 15
$\mathbf{V_{max}}$ (nMol.min^{-1}.mg^{-1}.)	7.89 ± 0.424 0.230 ± 0.05	21.45 ± 3.2 0.989 ± 0.03

The Direct spectrophotometric assay at 250 nm was used in all cases. Values quoted are the means + standard errors from the curve fits obtained from two or more separate determinations (as shown in brackets).

Figs. 4 and 5 illustrate the determination of the Michaelis constants for the oxidation of FCE 25692 and the parent amine benzylamine by rat liver MAO-B, respectively, and are taken as being representative of the other enzyme form used. The progress curves for the inhibition of monoamine oxidase-B from both species used by FCE 25692 would be consistent with this compound acting as both a substrate and as an irreversible mechanism-based inhibitor of the enzyme (suicide substrate) according to the mechanism shown in Scheme-1.

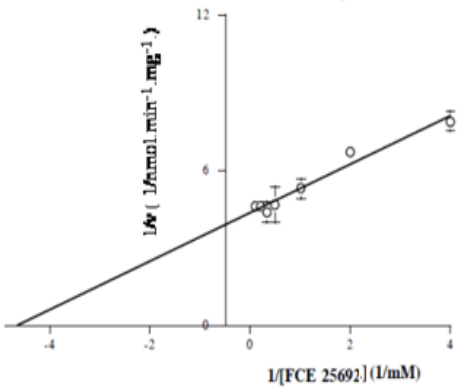


Figure 4. Determination of the Michaelis Constant for the Oxidation of FCE 25692 by Rat Liver Mitochondrial MAO-B.

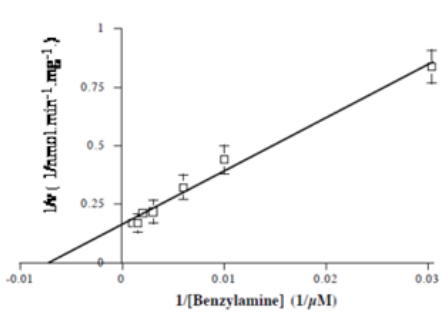
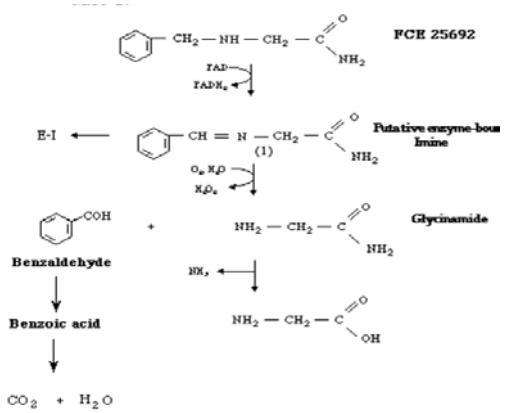


Figure 5. Determination of the Michaelis Constant for the Oxidation of Benzylamine by Rat Liver Mitochondrial MAO-B



Scheme 1. The mechanism of FCE 25692 oxidation by MAO-B as would be expected since FCE 25692 acts as both a 'suicide' substrate and specific MAO-B inhibitor. E-I represents the irreversibly inhibited species.

The partition ratio (r) was calculated by determining the amount of product formed at complete inactivation [P∞] for different enzyme inhibitor ratios. The absolute enzyme concentrations (eo) were determined as described in (Ben Ramadan *et al.*, 2012). The values obtained for the partition ratio of rat and ox liver mitochondrial MAO-B were 817 and 2120, respectively (Table 2.).

Table 2. The Kinetic Parameters for the interactions of the two Preparations of Monoamine Oxidase-B with FCE 25692.

Enzyme preparation	Rat Liver Mitochondria	Ox Liver Mitochondria
t1/2 (min)	21.5±3.5	34.0 ± 9
r_	816.7±121	2120 ± 55
K'(mM)	1.622±0.02	1.598± 0.01
Kin (min-1)	0.033±0.001	0.020±0.002

The values were determined by the procedure of Waley (1980 &1985) as described in the text. Each value represents the mean ± S.E.M. For at least 3 replicates

3.2. The Kinetic Parameters for the Mechanism Based Interactions of MAO-B from Both Species with FCE 25692

Figs. 6a and 7a show a series of reaction-progress curves for rat liver mitochondrial MAO-B and ox liver mitochondrial MAO-B, respectively with different concentrations of FCE 25692 but with the enzyme: FCE 25692 ratios kept constant. These curves were analyzed by the procedure of Waley (1980 &1985), as described earlier and a plot of $I_o.t_{1/2}$ versus the initial FCE 25692 concentration (I_o) were constructed for each (Figs.6b and 7b, respectively). From these the values of the apparent K_m (K') and the inactivation constants (k_{in}) obtained and they were (K') 1.60, and 1.62 mM and (k_{in}) 0.020, and 0.033 min^{-1}for ox liver mitochondrial MAO-B and rat liver mitochondrial MAO-B, respectively, as shown in Table 3.

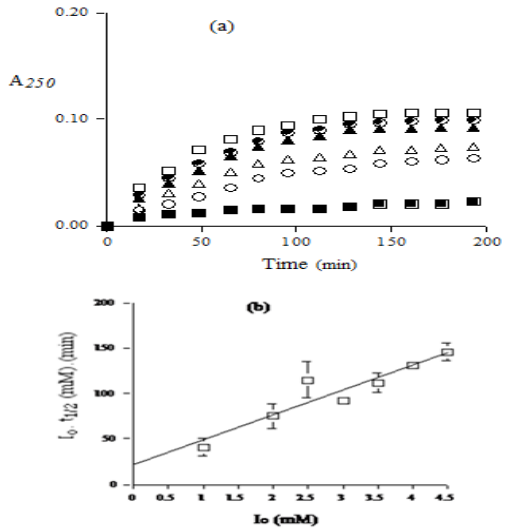


Figure 6. Determination of the Kinetic Parameters of Rat Liver Mitochondrial MAO-B towards FCE 25692.

a) Time Courses of Oxidation of FCE 25692 by Rat Liver Mitochondrial MAO-B at Different Concentrations of Each: The reactions of rat liver mitochondrial MAO-B and FCE 25692 (■) 1, (⬭) 2, (△) 2.5, (▲) 3, (◕) 3.5, and (□) 4 mM, were followed spectrophotometrically at 250 nm. The ratio between the enzyme concentration and FCE 25692 concentration was fixed. The points shown are the results from six representative experiments.

b) Half-time Plot for the Mechanism Based Inhibition of Rat liver Mitochondrial Monoamine oxidase-B by FCE 25692: The plot of $[I_o].t_{1/2}$ against $[I_o]$ for a series of experiments as shown above (a) in which $e_o/[I_o]$ was kept fixed where e_o is the initial concentration of rat liver mitochondrial MAO-B and I_o is the initial concentration of FCE 25692 .The points shown are the mean values ± range from two separate experiments.

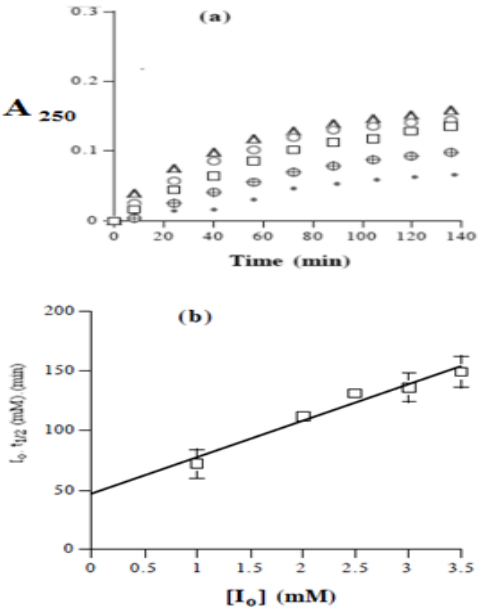


Figure 7. Determination of the Kinetic Parameters of Ox Liver Mitochondrial MAO-Btowards FCE 25692

a) Time Courses of Oxidation of FCE 25692 by Ox Liver Mitochondrial MAO-B at Different Concentrations of Each: The reactions of ox liver mitochondrial MAO-B and FCE 25692 , (*) 1, (⊕) 2, (□) 2.5, (⬭) 3, and (△) 3.5mM, were followed spectrophotometrically at 250 nm. The ratio between the enzyme concentration and FCE 25692 concentration was fixed. The points shown are the results from five representative experiments.

b) Half-time Plot for the Mechanism Based Inhibition of Ox liver Mitochondrial Monoamine oxidase-B by FCE 25692: The plot of [Io].t 1/2 against [Io] for a series of experiments as shown above (a) in which eo /[Io] was kept fixed where eo is the initial concentration of ox liver mitochondrial MAO-B and Io is the initial concentration of FCE 25692 . The points shown are the mean values ± range from two separate experiments.

Table 3. A Comparison of the kinetic Parameters for FCE 25692 as a Substrate and an Inhibitor of the Monoamine Oxidase-B Preparations

Enzyme preparation	Rat Liver Mitochondria	Ox Liver Mitochondria
Km (mM)	0.229	0.920
Kcat (min-1)	27.3	42.4
kcat/Km(min-1.mM-1)	118.7	46.1
K' (mM)	1.622	1.598
Kin (min-1)	0.033	0.020
kin/K' (min-1.M-1)	20.6	12.8

The catalytic constant kcat (maximum velocity / enzyme concentration) = Kin.r.

4. Discussion

The progress curves for the inhibition of MAO-B by FCE 25692 would be consistent with the compound acting as both a substrate and time-dependent irreversible inhibitor of the enzyme. This shows that the mechanism of action of FCE 25692 is similar to the mechanism of action of milacemide (Ben Ramadan et al., 2012) which acts as both a substrate and inhibitor of MAO-B and to that described for MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) (Tipton et al.,1986) and 3-{4-[(3-chlorophenyl)methoxy]phenyl}-5-[(methylamino)methyl]-2-oxazolidinone methanesulphonate (MD 780236) (Tipton et al., 1983a). The reaction pathway of FCE 25692 oxidation by MAO-B, would be expected as shown in Scheme-1. FCE 25692 is oxidized by MAO-B to form an imine intermediate. This intermediate can react with MAO-B to form a covalently bound intermediate that inactivates the enzyme or is hydrolyzed to liberate glycinamide and benzaldehyde. The latter product is then oxidized to benzoic acid. Benzoic acid is readily oxidized to carbon dioxide and water, whereas, glycinamide is broken down by amidase activities in brain microsomes to glycine. The detailed reaction mechanism may be more complex than this with intermediate radical intermediates being the actual inhibitory species. Unlike the behavior of milacemide (Ben Ramadan et al., 2012), the present

study did not show a large species differences between rat and ox liver MAO-B and the progress curves of the reactions, as monitored using the direct spectrophotometric assay, were seen to depart from linearity after few minutes with both MAO-B preparations studied. The values of the half-lives (Table 2) confirmed this; the t1/2value for ox preparation was a little larger than that for the enzyme from rat liver. The high values of the partition ratios Table (2) for the preparations used indicate that FCE 25692 functions as a better substrate for the enzyme MAO-B than as a mechanism-based inhibitor. In accordance with the behavior of milacemide (Ben Ramadan et al., 2012), the partition ratio for ox liver MAO-B (2120) is considerably higher than that of the enzyme from rat liver (817):

The progress curves for the inhibition of MAO-B from the two preparations showed that FCE 25692 was a better inhibitor for rat preparation and this is in agreement with the IC50 values previously reported by (O'Brien et al.,1994). FCE 25692 was shown to have a 4-fold lower Km value as a substrate for rat liver MAO-B (229 µM) than for the ox liver enzyme (920 µM). Thus, on these criteria, FCE 25692 is a better substrate for rat liver MAO-B than ox liver MAO-B. However, the kcat values (Table 3) obtained for mitochondrial enzymes from ox liver (42.0min-1) were somewhat higher than that for the rat liver mitochondrial MAO-B (27.3min-1). These differences are reflected in the specificity constants (kcat/Km values) which are the most useful indicators of the substrate specificity of an enzyme (see Cornish-Bowden, 1974). The turnover numbers (kcat) and the kcat/Km values are compared with the inhibition specificity constants (Kin /K') in Table 3. These values confirmed the fact that FCE 25692 is a better substrate for rat liver MAO-Bthan for ox liver MAO-B with kcat/Km values of (118 and 46.1 min-1.mM-1, respectively). Though the kinvalues were not much different, they show that FCE 25692 is somewhat a better inhibitor for ox liver MAO-B than rat liver MAO-B. The selectivity of FCE 25692 and milacemide as substrates for MAO-B may be related to the lipophilicity of these compounds, which is a common property of several MAO-B substrates. It is clear that milacemide is a much better substrate for MAO-B from rat and ox preparations (Ben Ramadan et al., 2012) than its benzylamine derivative, FCE 25692 in present studies. This could reflect the behavior of the precursor amines from which they were derived, since n-pentylamine, the parent amine of milacemide has a much lower Kmtowards MAO-B (Ben Ramadan et al., 2012) than benzylamine, the parent amine of FCE 25692; this difference may be an effect of the electronegative benzene ring of benzylamine. At low FCE 25692 concentrations the relative values of kin / K' (the inhibition specificity constant) for ox liver MAO-B was about half that for rat liver MAO-B. This is different than the inhibition specificity constants for milacemide (Ben Ramadan et al., 2012), where the value for the ox liver enzyme was found to be more than 6-times lower than that for the rat liver enzyme.

The reaction between rat liver mitochondria (600 µg) and FCE 25692 (2 mM) was followed spectrophotometrically at 250 nm using the direct assay. Upon completion of initial reaction, (a) at the point indicated by A, a further sample of FCE 25692 was added to raise the final concentration by 2 mM. In a second parallel experiment, at the point indicated by B (333µM) benzylamine was added. (b) At the point indicated by A more rat liver mitochondria was added to the reaction mixture and the reaction was followed further.

The initial rates of oxidation of FCE 25692 by rat liver mitochondria were measured, over the indicated range of concentrations. The results are presented as a double reciprocal Line weaver-Burk plot. Each point represents the mean value from three separate experiments.

The initial rates of oxidation of benzylamine by rat liver mitochondria were measured, over the indicated range of concentrations. The results are presented as a double reciprocal Line weaver-Burk plot. Each point represents the mean value from three separate experiments.

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References

Ben Ramadan Z, Tipton KF and Maria L. 2007. (N) - - Species Differences in the Selective Inhibition of Monoamine Oxidase (1-methyl-2-phenylethyl) hydrazine and its Potentiation by Cyanide. *Neurochem Res*, DOI 10.1007/s11064-007-9309-x.

Ben Ramadan Z and Tipton KF. 2012. Suicide Inhibition of Monoamine Oxidase from Different Species By Milacemide. *Jor J Biol Sci*, **5**: 269-278.

Cornish-Bowden A. 1974. Inhibitors and Activators in: Fundamentals of Enzyme Kinetics, (Cornish-Bowden, A., ed.), pp. 84-85, *Butterworths, London.*

Domino EF and Khanna SS. 1976. Decreased blood platelet MAO activity in unmedicated chronic schizophrenic patients. *The American Journal of Psychiatry*, **133**: 323-326.

Dostert P, Perarello P, Heidempergher F, Varasi M, Bonsignori A and Roncucci R. 1990. Preparation of a-(phenylalkylamino) carboxamides as drugs. *Eur Pat Appl*, **400**: 495.

Dostert P, Strolin Benedetti M and Tipton KF. 1991. New anticonvulsants with selective MAO-B inhibitoty activity. *Eur J Neuropsychopharmacol*, **1**: 317-319.

Janssens de Varebeke P, Cavalier R, David-Remacle M and Youdim MBH. 1988. Formation of the neurotransmitter glycine from the anticonvulsant milacemide is mediated by brain monoamine oxidase B. *J Neurochem*, **50**: 1011-1016.

Janssens de Varebeke P, Pauwells G, Buyse C, David-Remacle M, De Mey J, Roba J and Youdim MBH. 1989. The novel neuropsychotropic agent milacemide is a specific enzyme activated inhibitor of brain monoamine oxidase-B. *J Neurochem*, **53**: 1109-1116.

Kearney EB, Salach JI, Walker W H, Seng RL, Kenney W, Zeszotek E and Singer TP. 1971. The covalently bound flavin of hepatic monoamine oxidase. I. Isolation and sequence of a flavin peptide and evidence for binding at the 8a position. *Eur J Biochem*, **24:** 321-327.

MeyerJH, GinovartN,Boovariwala A,Sagrati S, HusseyD, Garcia A, Young T, Praschak-Rieder N, Wilson AA and Houle S . 2006. Elevated monoamine oxidase a levels in the brain: an explanation for the monoamine imbalance of major depression. *Arch Gen Psych*, **63**:1209–1216.

O'Brien EM, Dostert P, Paolo P and Tipton KF. 1994. Interactions of some analogues of the anti convulsant milacemide with monoamine oxidase. *Biochem Pharmacol*, **48**: 905-914.

Riederer P, LachenmayerLand Laux G. 2004.Clinical applications of MAO-inhibitors. *Curr Med Chem*, **11**: 2033–2043.

Salach JI. 1979. Monoamine oxidase from beef liver mitochondria: simplified isolation procedure, properties and determination of its cysteinylflavin content. *Arch Biochem Biophys*, **192**: 128-137.

Schildkraut JJ, Herzog JM, Orsulak PJ, Edelman SE, Shein HM and Frazier SH. 1976.Reduced platelet monoamine oxidase activity in a subgroup of schizophrenic patients. *Amer J Psych*, **133**: 438-440.

Shih JC and Chen K. 2004.Regulation of MAO-A and MAO-B gene expression. *Curr Med Chem*, **11**: 1995-2005.

Shulman KI, Herrmann N and Walker E S. 2013. Current Place of Monoamine Oxidase Inhibitors in the Treatment of Depression. *CNS Drugs*, **27**:789-797.

Tabor CW, Tabor H and Rosenthal SM. 1954. Purification of amine oxidase from beef plasma. *J Biol Chem*, **208**: 645-661.

Tipton KF. 1975. Monoamine oxidase in: Handbook of Physiology Section7: Endocrinology, vol. 6 (Blaschko, H., Sayers, G. & Smith, A.D., eds.), pp. 677-697, *American Physiological Society*, Washington.

Tipton KF and Youdim MBH. 1983. The assay of monoamine oxidase activity in: Methods in Biogenic Amine Research (Parvez, S., Nagatsu, T., Nagatsu, I. & Parvez, H., eds.), pp. 441-465, *Elsevier Press*, Amsterdam, New York and Oxford.

Tipton KF, Fowler CJ, McCrodden JM and StrolinBenedetti M. 1983a. The enzyme-activated irreversible inhibition of type-B monoamine oxidase by 3-(4-((3-chlorophenyl)methoxy) phenyl)-5((methylamino)methyl)-2-oxazolidinone methane sulphonate (compound MD 780236) and the enzyme-catalysed oxidation of this compound as competing reactions. *Biochem J*, **209**: 235-242.

Tipton KF, McCrodden JM and Youdim MBH. 1986. Oxidation and enzyme-activated irreversible inhibition of rat liver monoamine oxidase-B by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). *Biochem J*, **240**: 379-383.

Tipton KF. 1989. Monoamine oxidase inhibitors as antidepressants. In: Tipton KF and Youdim MBH. (eds) Biochemical and pharmacological aspects of depression. Taylor & Francis, London, pp 1–24.

Tipton KF, Boyce S, O'Sullivan J, DaveyGP and Healy J. 2004. Monoamine oxidases: certainties and uncertainties. *Curr Med Chem*, **11**: 1965-1982.

Waley SG. 1980. Kinetics of suicide substrates. *Biochem J*, **185**: 771-773.

Waley SG. 1985. Kinetics of suicide substrates. Practical procedures for determining parameters. *Biochem J*, **227**: 843-849.