J. Pharm. Pharmac., 1975, **27**, 682–691 *Received March* 26, 1975

Evidence for a clorgyline-resistant monoamine metabolizing activity in the rat heart

G. A. LYLES* AND B. A. CALLINGHAM

Department of Pharmacology, University of Cambridge, Medical School, Hills Road, Cambridge CB2 2QD, U.K.

When benzylamine was used as substrate, a component of the total monoamine oxidase (MAO) activity in the rat heart was found to be resistant to inhibition by clorgyline. The proportion of the total activity represented by this component, decreased as the rat grew. It was also inhibited by both semicarbazide and isoniazid but not by potassium cyanide. Inhibitor studies with MAO in subcellular fractions showed that this component was more concentrated in the microsomal and soluble fractions. However, it could not be concluded that the activity was entirely a soluble enzyme. Determination of quasi-Michaelis constants ("Km") for total benzylamine oxidizing activity revealed a high ("Km" of approximately 10^{-5}M) and low ("Km" of approximately 5×10^{-4}M) affinity component. The high affinity component was inhibited by semicarbazide and the low affinity component by clorgyline. In the presence of 10^{-3}M clorgyline, the high affinity component showed substrate inhibition at higher substrate concentrations. The possibility is discussed that the clorgyline-resistant activity is due to an amine-oxidizing activity distinct from mitochondrial MAO.

The amine oxidases are a group of enzymes differing from each other in a variety of ways, such as substrate specificity, inhibitor specificity and cellular localization (see Blaschko, 1974). The group includes such enzymes as connective tissue amine oxidases, plasma amine oxidases, diamine oxidases and mitochondrial monoamine oxidase (MAO). By the use of the selective irreversible inhibitor clorgyline, it has been suggested that the enzyme MAO exists in multiple forms, even within the same animal tissue. These forms have been designated enzyme A and enzyme B MAO according to their sensitivities towards the inhibitor (Johnston, 1968; Hall, Logan & Parsons, 1969). In the rat heart, we have previously reported that benzylamine metabolism seems to be mediated by both enzyme A and B (Lyles & Callingham, 1974). Furthermore, the relative proportions of these enzyme species change with age in the rat heart, such that the increasing specific activity of MAO that occurs with age (Horita, 1967), seems to be due to a selective increase in the activity of species A (Callingham & Lyles, 1975). During the course of these studies it was observed that there appeared to be a component of the total enzyme activity towards benzylamine as substrate that could not be inhibited, even by the higher clorgyline concentrations that would normally inactivate both species A and B. This present report describes an attempt to discover the nature of this clorgyline-resistant activity.

* G.A.L. is a Medical Research Council Scholar.

MATERIALS AND METHODS

Materials

The radioactive substrates, [^{3}H]tyramine and [^{14}C]benzylamine, used in the assay for MAO, were obtained from New England Nuclear GMBH, Dreieichain and ICN Pharmaceuticals (UK) Ltd., Hersham, Surrey, respectively.

Clorgyline hydrochloride (M & B 9302) was a gift from May & Baker Ltd., Dagenham, Essex. Isoniazid and semicarbazide were obtained from Sigma London, Kingston-upon-Thames, Surrey. Male albino Wistar rats were supplied by A. J. Tuck & Son, Rayleigh, Essex.

Methods

Rats were weighed and then killed by cervical dislocation. Hearts were removed, washed quickly in saline, blotted and the larger blood vessels dissected away. After weighing, each heart was homogenized in 0·001 M potassium phosphate buffer, pH 7·4 as a 1 to 10 (w/v) suspension of tissue in buffer, and then centrifuged at 600 *g* for 10 min. The supernatant resulting from this centrifugation was stored on ice for immediate MAO assay or deep frozen for longer storage.

Subcellular fractionation of the heart tissue was based on the method of Zak, Etlinger & Fischman (1970). Minced heart tissue was stirred at 0–4°C during several changes of a "relaxing" buffer containing KCl 0·1 M, $MgCl_2$ 5, EGTA 5, $Na_4P_2O_7$ 5 mM, pH 6·8. The relaxing buffer was then decanted and the muscle washed in a buffer containing sucrose 0·25 M, KCl 0·05 M, EGTA 5, $Na_4P_2O_7$ 1, MgCl 5 mM, pH 6·8.

The tissue was then homogenized in this buffer at a volume-to-weight ratio of 10:1 using a conical glass homogenizer. This homogenate was centrifuged at 800 *g* for 10 min. The supernatant was saved as the "low-speed supernatant" and several samples put aside for future assay. The bulk of this supernatant was then centrifuged at 9000 *g* for 10 min to sediment the mitochondria. The mitochondrial pellet was washed and resuspended in buffer and then recentrifuged first at 800 *g* for 10 min to remove contaminating debris and then subsequently at 9000 *g* for 10 min to resediment the mitochondria. The suspension made by resuspending this pellet, in buffer, was used as the mitochondrial fraction.

The supernatant resulting from the first sedimentation of mitochondria was subjected to a further centrifugation at 9000 *g* for 10 min. The supernatant was then decanted and centrifuged at 105 000 *g* for 60 min to produce a microsomal pellet. This pellet was resuspended in buffer and used as the microsomal fraction.

The supernatant resulting from this microsomal sedimentation was saved and used as the "high-speed supernatant".

Details of the radiochemical assay used for MAO have been previously described (Callingham & Laverty, 1973). Succinic dehydrogenase was assayed spectrophotometrically by the method of Green, Mii & Kohout (1955) using 2,6-dichlorophenolindophenol.

Protein contents were estimated by the micro-biuret method of Goa (1953).

RESULTS

Effects of inhibitors on the oxidation of benzylamine in the rat heart at different ages

Inhibition studies were carried out by preincubation of normal homogenate aliquots with the inhibitor for 20 min at 37° before addition of substrate for the assay of MAO.

Clorgyline was found to inhibit enzyme activity, but the percentage of the total enzyme activity that was inactivated by clorgyline, increased as the rat grew older. Semicarbazide and isoniazid were also found to be inhibitors, but here the percentage of the total activity that was inactivated by these drugs decreased as the rats grew older. Cyanide was found to have no inhibitory effect at all. Combination of clorgyline and semicarbazide resulted in almost complete inhibition of enzyme activity. These results are shown in Table 1.

Table 1. *Effects of inhibitors on benzylamine metabolism in the rat heart at different ages.* Each value is expressed as a percentage of the control activity and represents the mean of duplicate determinations on three different homogenates at each body weight range ± s.e. of the ratio. n.d. denotes not determined.

Mean body weight (g)	Inhibition % Semicarbazide (10^{-3} M)	Isoniazid (10^{-3} M)	KCN (10^{-2} M)	Clorgyline (5×10^{-4} M)	Clorg. (5×10^{-4} M) + Semicarb (10^{-3} M)
36	45·8 ± 9·5	41·2 ± 4·0	0	61·3 ± 1·1	89·7 ± 1·1
220	29·3 ± 5·4	21·6 ± 5·9	0	90·1 ± 0·5	98·0 ± 0·4
546	18·1 ± 0·4	17·1 ± 10·5	0	97·0 ± 0·7	n.d.

Distribution of MAO in subcellular fractions of rat heart tissue

Subcellular fractions of rat heart tissue were prepared as described in Methods. The animals used for this preparation weighed between 140 and 180 g with a mean body weight and heart weight of 167 g and 595 mg respectively. Table 2 shows the specific activity of MAO, measured with tyramine and benzylamine as substrates, in each of the fractions prepared. Also included for comparison with MAO is the specific activity of succinic dehydrogenase in the mitochondrial, microsomal and high-speed supernatant fractions. The recoveries shown for these enzymes are percentages of the total activity recovered in the mitochondrial, microsomal and high-speed supernatant fractions combined.

MAO was found to have a higher specific activity in the microsomal fraction than in the mitochondrial fraction. Succinic dehydrogenase activity is an inner mitochondrial membrane enzyme marker (Pycock & Nahorski, 1971). Although it was present in the microsomal fractions, in these experiments which would suggest some mitochondrial contamination, the succinic dehydrogenase showed a lower specific activity in the microsomes than in the mitochondria. The relative increase in MAO activity within the microsomes is consistent with two interpretations. First, MAO may truly be in

Table 2. *Subcellular distribution of MAO and SDH in fractions from rat heart (all assays were performed in duplicate).*

Fraction	Monoamine oxidase				Succinic dehydrogenase	
	Tyramine		Benzylamine			
	Specific activity [nmol (mg protein)$^{-1}$h^{-1}]	Recovery (%)	Specific activity [nmol (mg protein)$^{-1}$h^{-1}]	Recovery (%)	Specific activity [nmol (mg protein)$^{-1}$min^{-1}]	Recovery (%)
Low-speed supernatant	113·4		7·9			
Mitochondria	129·4	40	8·9	33	27·2	61
Microsomes	187·8	52	13·1	44	14·8	28
High-speed supernatant	8·0	8	1·8	23	1·6	11

association with the endoplasmic reticulum, a suggestion proposed by de Champlain, Mueller & Axelrod (1969). Alternatively, during the harsh disruptive procedures needed to homogenize heart tissue, mitochondrial outer membranes may be separated from the rest of the mitochondrion and sediment within the microsomal fraction thus causing the relative increase in MAO activity in this way (Jarrott & Iversen, 1968).

Although the MAO had a low specific activity within the high-speed supernatant, its recovery using benzylamine as substrate was fairly high (23%) within this fraction. This was in contrast to a very low recovery (8%) in this fraction if tyramine was used as substrate. Assay of SDH showed that there was still a very small mitochondrial contamination within this supernatant.

Inhibition of MAO in subcellular fractions by clorgyline

Inhibition curves for MAO, using benzylamine as substrate, in each of the subcellular fractions, were obtained by incubation with serial dilutions of clorgyline. These results are shown in Fig. 1.

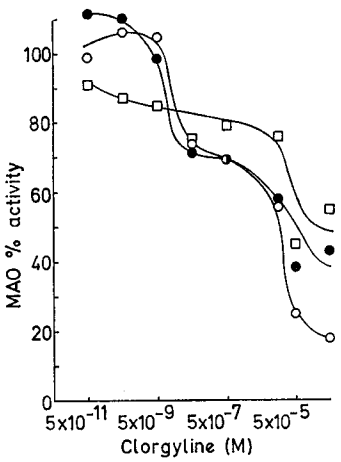

FIG. 1. Effect of clorgyline upon the *in vitro* activity of MAO in subcellular fractions of rat heart. Benzylamine was used as substrate. Each point is the mean of duplicate assays at each inhibitor concentration; activities are expressed as percentages of activity in the corresponding untreated fraction. ○—○ mitochondria; ●—● microsomes; □—□ high speed supernatant.

In each of the fractions, inhibition of enzyme activity showed a biphasic nature with a plateau region. Furthermore, the position of this plateau agreed well with previously described inhibition plots obtained when clorgyline was used to inhibit the cardiac MAO in rats of similar body weight (Lyles & Callingham, 1974; Callingham & Lyles, 1975). The most striking feature of these curves was the residual activity which was not inhibited by the highest concentration of clorgyline used (5×10^{-4} M). The proportion of residual activity was greatest in the high-speed supernatant fraction and least in the mitochondria.

Effects of other inhibitors on MAO in subcellular fractions

Table 3 shows the effects of a number of other inhibitors at a single dose upon MAO activity in the various subcellular fractions. Both benzylamine and tyramine were used as substrates in these experiments. Semicarbazide was clearly an inhibitor of

Table 3. *Effect of inhibitors on MAO in subcellular fractions from rat heart.* Each value is expressed as a percentage of the control activity. Assays were performed in duplicate on fractions obtained from a pooled homogenate of 18 hearts as described in the text. Benz. = Benzylamine, Tyr. = Tyramine.

Fraction	Inhibition % Semicarbazide (10^{-3} M)		Isoniazid (10^{-3} M)		KCN (10^{-3} M)		Clorgyline (10^{-3} M)	
	Benz.	Tyr.	Benz.	Tyr.	Benz.	Tyr.	Benz.	Tyr.
Low-speed supernatant	32	8	4	0	0	0	70	98
Mitochondria	28	9	4	10	0	0	77	98
Microsomes	42	12	13	0	0	0	57	98
High-speed supernatant	43	0	0	0	0	0	45	98

benzylamine oxidizing activity, the percentage inhibition being greatest within the microsomal and high-speed supernatant fractions. However, there was still a fairly large inhibition of the mitochondrial fraction. Isoniazid showed a small inhibition of the activity and potassium cyanide was without effect. Clorgyline was most effective in the mitochondrial and least effective in the supernatant fraction.

With tyramine as substrate, semicarbazide and isoniazid were much less effective inhibitors, whereas almost complete inhibition of enzyme activity in all the fractions was seen with clorgyline.

Determination of quasi-Michaelis constant for MAO in the mitochondrial fraction using benzylamine

As a result of the low activity of rat heart MAO towards benzylamine, determination of initial reaction velocities at very low substrate concentrations (eg 6 μM) was almost impossible since one was then working at the limit of sensitivity of the assay. The net counts min^{-1} due to formation of radioactive product was little greater than the blank value of about 20 after an incubation period of 5 min. However, this problem could be surmounted by incubating the enzyme sample for a much longer time at 37° with substrate than was usually employed in normal enzyme assays. For this reason enzyme activity was assayed for a single 60 min incubation time rather than the 10, 20, 40 and 60 min incubation times used to generate progress curves. Although, after 60 min the reaction is no longer linear, sufficient extractable radioactivity in the metabolites is produced above blank values by this time to give a meaningful differentiation of enzyme activity at different substrate concentrations. It is then possible to use the accumulated radioactivity as an apparent rate of reaction, which should bear the same relation to the real initial velocities over the range of substrate concentrations employed. However, any V_{max} value produced in this way from a Lineweaver Burk plot of the results will have no true meaning but the quasi-Michaelis constant ("Km") obtained by this method should be close enough to the real Km for comparative evaluations.

"Km" values were thus determined for the enzyme in a mitochondrial fraction alone, and subsequently in the mitochondrial fraction in the presence of clorgyline (10^{-7} M) and also in the presence of semicarbazide (10^{-3} M). As is usual in studies with these inhibitors, the enzyme was preincubated with the inhibitors for 20 min at 37° before addition of substrate. Substrate concentrations used were 6·25, 12·5, 31·25, 62·5, 125, 250 μM, 1, 2, 5 mM. Figs 2 and 3 show the Lineweaver Burk plots obtained.

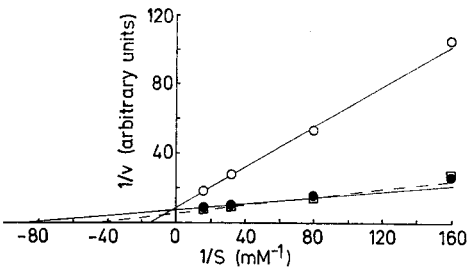

FIG. 2. Lineweaver Burk plots for rat heart mitochondrial MAO at low benzylamine concentrations (6·25, 12·5, 31·25, 62·5 μM) with and without the inhibitors clorgyline and semicarbazide. Each point represents the mean of duplicate determinations at each substrate concentration. "Km" values were derived from a computer program fitting the best staight line to the points by the method of least squares. ■- - -■ no inhibitor: "Km"=2·1 × 10^{-5}M; ●——● with clorgyline (10^{-7}M): "Km" = 1·1 × 10^{-5}M; ○——○ with semicarbazide (10^{-3} M): "Km" = 6·4 × 10^{-5}M.

The results indicated the presence of a high and low affinity component of benzylamine metabolizing activity. The high affinity component had a "Km" of approximately 10^{-5} M whereas the lower affinity component had a "Km" of approximately 5 × 10^{-4} M. Clorgyline seemed to have no effect on the higher affinity component, but altered the slope of the lower affinity component with a resultant change in the apparent V_{max} but no change in the "Km". This is consistent with non-competitive inhibition. It thus seems possible that this lower affinity component is the mitochondrial MAO which is inhibited by clorgyline. The concentration of clorgyline used was that which will selectively inhibit enzyme A activity but not enzyme B activity. In this case the "Km" derived in the presence of this concentration of clorgyline presumably represents the "Km" of enzyme B. However, since this value shows no change from the value in non-inhibited mitochondrial MAO, which represents a mixture of enzyme A and B, it seems likely that these two species have similar "Km"

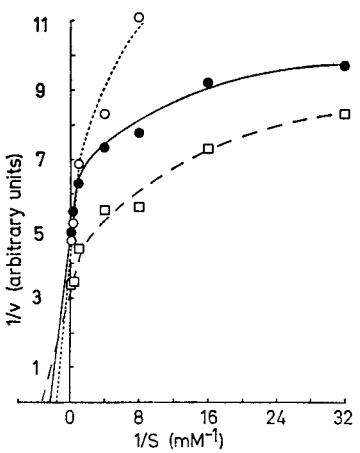

FIG. 3. Lineweaver Burk plots for rat heart mitochondrial MAO at high benzylamine concentrations (31·25, 62·5, 125, 250 μM, 1·0, 2·5, 5·0 mM) with and without the inhibitors clorgyline and semicarbazide. Each point represents the mean of duplicate determinations at each substrate concentration. "Km" values were derived from a computer program fitting the best straight line to the points at 1·0, 2·5, 5·0 mM by the method of least squares. □- - -□ no inhibitor: "Km" = 3·1 × 10^{-4}M; ●——● with clorgyline (10^{-7}M): "Km" = 4·6 × 10^{-4}M; ○- - -○ with semicarbazide (10^{-3}M): "Km" = 6·6 × 10^{-4}M.

values irrespective of whether they actually represent isoenzymes or not. In contrast, semicarbazide appeared to affect preferentially the high affinity component.

Similar experiments were performed using the low speed supernatant from rats weighing 100–110 g. In this case Lineweaver Burk plots were obtained in the presence and absence of 10^{-3} M clorgyline, a concentration that almost completely inhibits both enzyme A and B. These results are shown in Fig. 4.

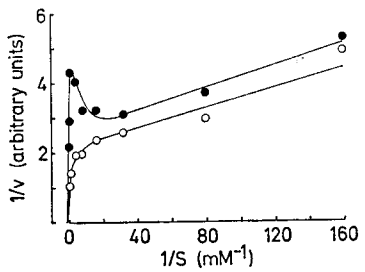

FIG. 4. Lineweaver Burk plots for rat heart low-speed supernatant MAO with and without clorgyline. Substrate concentrations ranged from 6·25 μM to 5·0 mM. Each point represents the mean of duplicate determinations at each substrate concentration. "Km" values were determined from a computer program fitting the best straight line to the linear portions of the plots. (High affinity component: values of S between 6·25 and 31·25 μM. Low affinity component: values of S between 1·0 and 5·0 mM). ○——○ no inhibitor: "Km" high affinity = $6{\cdot}4 \times 10^{-6}$M, low affinity = $6{\cdot}8 \times 10^{-4}$M; ●——● with clorgyline (10^{-3}M): "Km" high affinity = $6{\cdot}0 \times 10^{-6}$M; low affinity = $1{\cdot}8 \times 10^{-3}$M.

In the absence of inhibitor a downward curving plot was obtained suggesting the presence of high and low affinity components. In the presence of clorgyline the resulting plot indicated that the high affinity component was inhibited by high concentrations of substrate. This effect had previously been obscured by the low affinity component. However, even in the presence of this high concentration of clorgyline a very small amount of this low affinity component still remained, but this did not interfere with the demonstration of substrate inhibition of the high affinity component.

In these experiments there was an indication, shown by the elevation of the Lineweaver Burk plot that this very high concentration of clorgyline produced a small inhibition of the high affinity component.

It has not been possible to make an accurate measurement of the Km values of the enzyme components due to the complex nature of the plots obtained and the inability completely to separate the various components from each other. However, care was taken to derive the "Km" values from linear regions of the plots.

Effects of semicarbazide on the inhibition of MAO by clorgyline

In these experiments low speed supernatant fractions from rat hearts of animals weighing 100–110 g were used. Clorgyline inhibition curves, using benzylamine as substrate, were obtained as described earlier, with and without the addition of 10^{-3} M semicarbazide. When this latter compound was present it was so for both the preincubation and incubation periods. These results are shown in Fig. 5.

The addition of semicarbazide to the incubation mixture completely eliminated all residual MAO activity and produced a roughly constant addition to the inhibition of MAO at all concentrations of clorgyline used. In the absence of semicarbazide, very

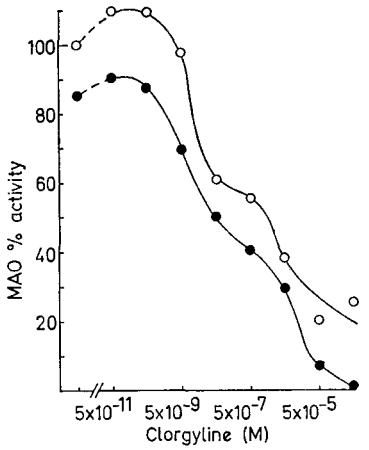

FIG. 5. The effect of clorgyline upon *in vitro* activity of MAO in a low-speed supernatant from rat heart with and without semicarbazide. Benzylamine was used as substrate. Each point represents the mean of duplicate determinations at each concentration of clorgyline; activities are expressed as percentages of activity in the non-inhibited low-speed supernatant. ○——○ no semicarbazide; ●——● semicarbazide (10^{-3} M).

low concentrations of clorgyline (5×10^{-11} to 5×10^{-10} M) frequently caused a slight but not significant increase in MAO activity. No explanation at present can be offered for this effect.

DISCUSSION

The oxidation of benzylamine by MAO in the rat heart appears to be brought about by more than one enzymic activity. We have previously reported that the use of the irreversible inhibitor clorgyline shows that benzylamine is metabolized by two activities that were originally defined as enzyme A and B on the basis of their sensitivities to clorgyline (Johnston, 1968), whereas tyramine appears to be metabolized by enzyme A (Lyles & Callingham, 1974). In a number of other tissues such as rat vas deferens (Jarrott & Iversen, 1971) and rat brain (Hall & others, 1969) benzylamine has always shown a single sigmoid inactivation curve with clorgyline, suggesting that it is metabolized by enzyme B alone. As far as we know, the rat heart is the only tissue so far studied in which a biphasic curve of this nature is shown with benzylamine. In this context it is interesting to note that β-phenylethylamine, which is reported to be a specific substrate for enzyme B MAO in rat brain (Yang & Neff, 1973) is metabolized only by enzyme A in the rat heart as also are 5-HT, tryptamine, kynuramine and dopamine (unpublished observations).

It is well documented that mitochondrial MAO contains flavine adenine dinucleotide as its cofactor (Youdim & Sourkes, 1972) and that irreversible inhibitors which contain an acetylenic triple bond in their structure, such as pargyline and clorgyline interact covalently with the cofactor (Oreland, Kinemuchi & Yoo, 1973; Chuang, Patek & Hellerman, 1974). When using benzylamine as a substrate, we consistently observed a proportion of the total activity which was not inhibited by the higher concentrations of clorgyline that would normally inactivate enzyme A and B MAO. This clorgyline-resistant activity represented a greater proportion of the total activity in young rats, whereas this proportion decreased in the older rats. The specific activity

of MAO increases with age in the rat heart (Horita, 1967), and we have previously suggested that this increase is mediated specifically by a change in the enzyme A component of the MAO activity which gradually overwhelms the enzyme B activity as the rat ages (Callingham & Lyles, 1975). The present results indicate that this clorgyline-resistant activity also diminishes in proportion as a consequence of the increasing enzyme A activity.

Benzylamine is a substrate for a number of other amine oxidase-like enzymes which use pyridoxal phosphate and copper as their cofactors. The pyridoxal phosphate moiety renders these enzymes sensitive to carbonyl reagents such as semicarbazide. In the present results, part of the benzylamine metabolizing activity was inhibited by semicarbazide and to a lesser extent, by isoniazid. However, cyanide, which is an inhibitor of plasma amine oxidases had no effect on activity. Consequently, we can exclude the possibility that the residual activity is derived from contaminating blood trapped within the tissue before homogenization. In agreement with this is our previous inability to detect significant benzylamine metabolizing activity in rat blood (Lyles & Callingham, 1974).

Subcellular distribution studies showed that in agreement with previous workers, a considerable part of the recovered MAO activity in the rat heart is found in the microsomal fraction (de Champlain & others, 1969). In the present studies, both benzylamine and tyramine were used as substrates. With benzylamine in particular, a moderately large activity was recovered in the high-speed supernatant. In all the fractions studied, clorgyline inhibition curves were biphasic in shape using benzylamine, suggesting the presence of mitochondrial MAO in each fraction. However, clorgyline-resistant activity was also apparent in each fraction, and represented the greatest proportion within the high speed supernatant. It is difficult at this stage to suggest that the residual activity is entirely a soluble enzyme since significant proportions were found in the mitochondria and microsomes. However, it is possible that these fractions contain membrane components that have resealed around cytoplasm during their preparation. Alternatively, MAO is believed to be synthesized on the endoplasmic reticulum before being transported to the outer mitochondrial membrane (Erwin & Simon, 1969), and it is not known at present whether this newly-formed MAO has the same inhibitor specificity, substrate specificity or cofactor requirements as MAO incorporated within the mitochondrion. In this context, Callingham & Laverty (1973) observed that the increase in specific activity of MAO in the rat heart which follows adrenalectomy became evident at an earlier time after surgery if benzylamine was used as substrate than with tyramine. It is tempting to speculate that this represented early synthesis of MAO before incorporation into the mitochondria.

Determination of Michaelis constants of the MAO within the mitochondrial fraction clearly demonstrated the presence of two components which can metabolize benzylamine. The higher affinity component appeared to be inhibited by semicarbazide whereas the lower affinity component was inhibited by clorgyline. Furthermore, this latter component appeared to be a mixture of enzyme A and B MAO; these activities seem to have similar if not identical values for "Km".

From Fig. 4 it would seem likely that high concentrations of substrate will inhibit the high affinity component, but this effect is only seen when the low affinity enzymes A and B have largely been eliminated by 10^{-3} M clorgyline. However, this concentration of clorgyline does produce some inhibition of the high affinity component, but at present it is impossible to speculate upon the type or mechanism of the inhibition

produced. No detectable inhibition of this component was observed when experiments using 10^{-7} M clorgyline were performed on the mitochondrial fraction. The inhibition curves resulting from the combined use of clorgyline and semicarbazide would seem to indicate that no further major component remains to contribute to the total activity.

Considered as a whole, these results using benzylamine show a strong similarity to those of Coquil, Goridis & others (1973) who showed a component of rat artery MAO which was sensitive to inhibition by carbonyl reagents such as semicarbazide, but was resistant to clorgyline and cyanide. This activity appeared to be concentrated within the high-speed supernatant. However, these workers used tyramine as their substrates. In our results, the clorgyline-resistant activity with tyramine as substrate represented a very small proportion of the total activity. Nonetheless, although the hearts used in our work had the larger blood vessels dissected away before homogenization, it is likely that vascular elements would still be present within the homogenate. In particular, the heart, besides containing muscular tissues, also contains a considerable quantity of connective tissue cells (Zak, 1974). Amine oxidizing activities, which seem to differ from classical MAO have been localized in a number of other connective tissues (see Blaschko, 1974 for review) and it thus remains a strong possibility that these are the source of some or all of the clorgyline-resistant MAO activity in the rat heart.

Acknowledgements

We thank the British Heart Foundation for a generous grant in support of this work.

REFERENCES

BLASCHKO, H. (1974). *Rev. Physiol. biochem. Pharmac.*, **70**, 83–148.

CALLINGHAM, B. A. & LAVERTY, R. (1973). *J. Pharm. Pharmac.*, **25**, 940-947.

CALLINGHAM, B. A. & LYLES, G. A. (1975). *Br. J. Pharmac.* **53**, 458–459P.

DE CHAMPLAIN, J., MUELLER, R. A. & AXLEROD, J. (1969). *J. Pharmac. exp. Ther.*, **166**, 339–345.

CHUANG, H. Y. K., PATEK, D. R. & HELLERMAN, L. (1974). *J. biol. Chem.*, **249**, 2381–2384.

COQUIL, J. F., GORIDIS, C., MACK, G. & NEFF, N. H. (1973). *Br. J. Pharmac.*, **48**, 590–599.

ERWIN, V. G. & SIMON, R. J. (1969). *J. pharm. Sci.*, **58**, 1033–1036.

GREEN, D. E., MII, S. & KOHOUT, P. M. (1955). *J. biol. Chem.*, **217**, 551–567.

GOA, J. (1953). *Scand. J. clin. lab. Inv.*, **5**, 218–222.

HALL, D. W. R., LOGAN, B. W. & PARSONS, G. H. (1969). *Biochem. Pharmac.*, **18**, 1447–1454.

HORITA, A. (1967). *Nature, Lond.*, **215**, 411–412.

JARROTT, B. & IVERSEN, L. L. (1968). *Biochem. Pharmac.*, **17**, 1619–1625.

JARROTT, B. (1971). *J. Neurochem.*, **18**, 7–16.

JOHNSTON, J. P. (1968). *Biochem. Pharmac.*, **17**, 1285–1297.

LYLES, G. A. & CALLINGHAM, B. A. (1974). *J. Pharm. Pharmac.*, **26**, 921–930.

ORELAND, L., KINEMUCHI, H. & YOO, B. Y. (1973). *Life Sci.*, **13**, 1533–1541.

PYCOCK, S. J. & NAHORSKI, S. R. (1971). *J. mol. cell. Cardiol.*, **3**, 229–241.

YANG, H. Y. T. & NEFF, N. H. (1973). *J. Pharmac. exp. Ther.* **187**, 365–371.

YOUDIM, M. B. H. & SOURKES, T. L. (1972). In: *Monoamine Oxidases—New Vistas. Advances in Biochemical Psychopharmacology*, Vol. 5, pp. 45–53, Editors: Costa, E. & Sandler, M. New York: Raven Press.

ZAK, R., ETLINGER, J. & FISCHMAN, D. A. (1970). *Excerpta Medica*, International Congress Series No. 240—Research in Muscle Development and the Muscle Spindle, pp. 163–175.

ZAK, R. (1974). *Suppl. to Circ. Res.*, **34/35**. II. 17–26.