Biochemical Pharmacology, Vol. 23, pp. 2983–2990. Pergamon Press, 1974. Printed in Great Britain.

MONOAMINE OXIDASE INHIBITORS AND OTHER DRUGS AS INHIBITORS OF DIAMINE OXIDASE FROM HUMAN PLACENTA AND PIG KIDNEY

M. JAMES C. CRABBE and WILLIAM G. BARDSLEY

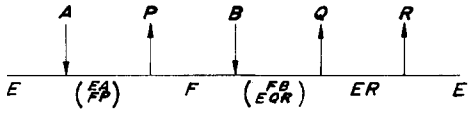
Department of Obstetrics and Gynaecology, University of Manchester, at St. Mary's Hospital, Whitworth Park, Manchester M13 0JH, England

(*Received* 8 *February* 1974; *accepted* 19 *March* 1974)

Abstract—A study of the effect of nine monoamine oxidase (MAO) inhibitors on purified diamine oxidases (DAO) from human placenta and pig kidney is reported, together with the effect of 32 drugs commonly used in pregnancy on purified human placental diamine oxidase. Values of inhibitor constants, calculated from the slopes and intercepts of double-reciprocal plots are given, together with ΔG° values for the enzyme–inhibitor interactions. From the results, we conclude that compounds once considered as specific monoamine oxidase inhibitors can no longer be thought of in this light, and that some of the drugs often used during pregnancy are potent *in vitro* inhibitors of human placental diamine oxidase.

DURING a study of the effects of substrate analogues as reversible inhibitors of diamine oxidase (histaminase, pyridoxal phosphate dependent amine oxidase E.C.N. 1.4.3.6) from pig kidney[1] and human placenta,[2] it was noticed that certain compounds known collectively as MAO inhibitors[3] were potent inhibitors of these DAOs.[2] It has been supposed that inhibition of DAO enzymes by these agents was not appreciable and that a distinction between MAO and DAO could be made by the use of MAO inhibitors.[4] A comprehensive study of this phenomenon is now reported. Also, since it is believed that placental DAO has a protective function in the placenta during pregnancy,[5] we have undertaken a close study of drugs commonly used in pregnancy to see if any of these adversely affect the DAO reaction.

Diamine oxidase catalyses the reaction between two substrates *A* and *B* (diamine and O_2) and three products, *P*, *Q* and *R*, (an amino-aldehyde, H_2O_2 and NH_3). We have isolated and purified this enzyme from pig kidney[6] and human placenta,[7] and kinetic analysis shows each to have a Ping Pong Bi Ter mechanism of the type



with two stable enzyme forms (*E* and *F*).

MATERIALS AND METHODS

Human placental diamine oxidase was purified by the method of Bardsley *et al.*[7] After column chromatography, the purest fraction had a sp. act. of 0·9 international units of enzyme/mg protein (1 international unit of enzyme defined as that

amount of enzyme which catalyses the oxidation of 1 μmole of substrate min^{-1} at 20°).

Pig kidney diamine oxidase was prepared according to the method of Bardsley *et al.*[6] the purified enzyme having a sp. act. of 1·4.

The inhibitory potency was determined using initial enzyme rates as described previously for the effect of substrate analogues on pig kidney[1] and human placental[2] diamine oxidases. All experiments were conducted at 20° in 0·05 M potassium phosphate buffer, pH 7·0, in air, in a final volume of 1 ml, containing appropriate concentrations of substrate and inhibitor. Change in E_{250} was measured as previously described[6] using *p*-dimethylaminomethylbenzylamine as substrate. The drugs used in pregnancy were proprietary products obtained from the Pharmacy Department, St. Mary's Hospital, Manchester.

Interpretation of the inhibition experiments. Since double reciprocal plots were linear with the concentration of *B* (oxygen) fixed, we can represent the uninhibited reaction by

$$\frac{1}{v} = m\left(\frac{1}{A}\right) + c$$

where the slope *m* is Ka/V and the intercept *c* is

$$\frac{1}{V}\left(1 + \frac{K_b}{B}\right).$$

Combination of inhibitor with enzyme form *E* according to

$$E + I = EI$$

and

$$EI + I = EI_2$$

will give slope effects (competitive inhibition) with

$$K_{i\ \text{slope}\ 1} = \frac{(E)(I)}{(E\,I)}$$

and

$$K_{i\ \text{slope}\ 2} = \frac{(E\,I)(I)}{(E\,I_2)}.$$

Intercept effects result from the combination of inhibitor with enzyme form *F* according to

$$F + I = FI$$

and

$$FI + I = FI_2$$

giving uncompetitive inhibition with

$$K_{i\ \text{intercept}\ 1} = \frac{(F)(I)}{(F\,I)}$$

and

$$K_{i\ \text{intercept 2}} = \frac{(F\,I)(I)}{(F\,I_2)}.$$

Where both slope and intercept effects are given, we have noncompetitive inhibition. The inhibition patterns observed in the present study were in all cases either linear or parabolic and no partial (hyperbolic) inhibition was seen.

Linear inhibition can be represented by

$$\frac{1}{v_i} = m\left(\frac{1}{A}\right)(1 + a\,I) + c\,(1 + \alpha\,I)$$

with m and c as before. I is the inhibitor concentration and values for $a(K_{i\ \text{slope 1}}{}^{-1})$ and $\alpha(K_{i\ \text{intercept 1}}{}^{-1})$ are obtained from replots of slopes and intercepts respectively against I. Standard free energies for the enzyme–inhibitor interactions are then obtained from

$$\Delta G^\circ = -RT \ln a \quad \text{or} \quad -RT \ln \alpha.$$

Parabolic inhibition can be represented by

$$\left[\frac{1}{v_i} = m\left(\frac{1}{A}\right)(1 + aI + bI^2) + c\,(1 + \alpha I + \beta I^2)\right]$$

where m, c, a, α and I are as previously described.

$$b = (K_{i\ \text{slope 1}} \quad K_{i\ \text{slope 2}})^{-1}$$

and

$$\beta = (K_{i\ \text{intercept 1}} \quad K_{i\ \text{intercept 2}})^{-1}.$$

When the slopes and intercepts are replotted as functions of I, parabolas result and the tangents to these parabolas are then replotted against I giving lines with slopes of $2bm$ or $2\beta c$ and vertical intercept am or αc. Standard free energies for the second enzyme–inhibitor interaction are calculated from

$$\Delta G^\circ = -RT \ln \frac{b}{a} \quad \text{or} \quad -RT \ln \frac{\beta}{\alpha}.$$

It is of some interest to relate the results represented by the parameters a, b, α and β to the percentage inhibition given by a certain inhibitor concentration in the presence of fixed concentration of substrate.[8] This is easily done by relating the percentage inhibition $P_{\%}$ to the velocity of the uninhibited reaction (v) to a multiple of the inhibited reaction (v_i) thus $v = nv_i$ giving

$$P_{\%} = \left(100 - \frac{100}{n}\right).$$

For linear inhibition we have

$$I = \frac{(n-1)\,[m\,(1/A) + c]}{[am\,(1/A) + \alpha c]}$$

giving 50 per cent inhibition for $(1/A) = 1$ when

$$I = \frac{m + c}{am + \alpha c}.$$

When the inhibition is parabolic, we have

$$I = \frac{-[am(1/A) + \alpha c] + \sqrt{\{[am(1/A) + \alpha c]^2 + 4(n-1)[bm(1/A) + \beta c][m(1/A) + c]\}}}{2[bm(1/A + \beta c]}$$

giving 50 per cent inhibition for $(1/A) = 1$ with

$$I = \frac{-(am + \alpha c) + \sqrt{[(am + \alpha c)^2 + 4(bm + \beta c)(m + c)]}}{2(bm + \beta c)}$$

RESULTS

Table 1 gives the inhibition data for MAO inhibitors with human placental DAO and pig kidney DAO.

Table 2 shows the type and degree of inhibition of placental DAO by drugs commonly used in obstetric practice.

TABLE 1. INHIBITION OF HUMAN PLACENTAL AND PIG KIDNEY DIAMINE OXIDASES BY TYPICAL MAO INHIBITORS

Inhibitor	Enzyme	Type of inhibition	Slope effect a	Slope effect $-\Delta G^\circ$ (K J mole^{-1})	Intercept effect α	Intercept effect $-\Delta G^\circ$ (K J mole^{-1})	P_{50} (mM)
Tranylcypramine	P	NC	11·44	22·76	*	*	0·114
	K	NC	2·85	19·38	16·66	23·68	0·0687
Harmine	P	NC	79·36	27·48	7·812	21·83	0·05
	K	NC	18·86	24·06	42·37	26·035	0·0255
Isocarboxazid	P	C	1·2	17·26	—	—	5·0
	K	C	1·14	17·2	—	—	5·7
Nialamide	P	NC	32·67	25·32	12·12	22·9	0·064
	K	NC	17·24	23·76	5·0	20·75	0·145
Iproniazid	P	NC	40	25·81	16	23·58	0·05
	K	C	30·3	25·21	—	—	0·214
Phenelzine	P	C	400	31·52	—	—	0·015
	K	NC	400	31·52	400	31·52	0·0025
Mebanazine	P	NC	32·25	25·29	40	25·81	0·0258
	K	NC	8·33	22·06	16	23·66	0·067
Isoniazid	P	C	19·23	24·03	—	—	0·312
	K	C	0·69	15·99	—	—	9·42
Pargyline	P	NC	12·82	23·05	23·25	24·5	0·051
	K	NC	12·04	22·9	1·428	17·7	0·818

* $a = 0{\cdot}7$; $-\Delta G_1^\circ = 15{\cdot}97$ K J mole^{-1}. $\beta = 66{\cdot}0$; $-\Delta G_2^\circ = 28{\cdot}05$ K J mole^{-1}.

P, human placental diamine oxidase; K, pig kidney diamine oxidase; NC, non-competitive inhibition; C, competitive inhibition. Values for P_{50} (50 per cent inhibition) are given for an amine concentration of 1 mM. The relationships between a, α, $-\Delta G^\circ$ and P_{50} are given in the text.

TABLE 2. INHIBITION OF HUMAN PLACENTAL DIAMINE OXIDASE BY DRUGS COMMONLY USED IN PREGNANCY*

Compound	Type of inhibition	Slope effect				Intercept effect				P_{50} (mM)
		a	$-\Delta G_1^\circ$ (K J mole^{-1})	b	$-\Delta G_2^\circ$ (K J mole^{-1})	α	$-\Delta G_1^\circ$ (K J mole^{-1})	β	$-\Delta G_2^\circ$ (K J mole^{-1})	
Aldomet	NC	3·125	19·6	—	—	2·38	18·9	—	—	0·411
Avertin	NC	0·56	15·4	—	—	0·188	12·7	—	—	4·68
Benzyl Penicillin	NC	1·25	17·37	—	—	0·045	9·3	—	—	7·89
Chlorothiazide	NC	30·3	13·9	—	—	38·46	25·7	—	—	0·026
Chlorpromazine	NC	10	22·4	112·5	22·7	0·272	13·66	28·1	28·13	0·159
Cyclophosphamide	NC	1·85	18·33	—	—	0·312	13·99	—	—	2·4
Diamorphine	NC	0·75	16·1	—	—	0·408	14·6	—	—	2·319
Diazepam	UC	—	—	—	—	0·909	16·59	104·5	28·39	0·097
Frusemide	NC	25·0	24·6		—	50·0	26·3	—	—	0·0207
Guanethidine	NC	11·1	22·7	—	—	4·35	20·4	—	—	0·208
Haloperadol	NC	100	28·05	—	—	4·54	20·5	—	—	0·09
Hydralazine	NC	62500	43·7	—	—	4166	37·1	—	—	0·000123
Isoxsuprine	NC	40·0	22·3	—	—	0·36	14·3	—	—	0·328
Pentolinium	NC	5·55	21·0			0·36	14·3	—	—	1·4
Perphenazine	NC	0·125	11·76	3·43	24·92	1·8	18·28	68·0	25·68	0·113
Prochlorperazine	NC	50·0	26·36	750	23·45	0·136	11·97	15·5	28·38	0·0995
Promazine	NC	1·25	17·37	181·3	28·95	0·36	14·34	43·6	28·5	0·1335
Promethazine	NC	1·25	17·37	250	29·73	0·863	16·47	41·1	26·25	0·1265
Propranolol	NC	2·12	18·6	—	—	0·63	15·7	—	—	1·368
Protoveratrine	NC	303	30·75	—	—	434·7	31·63	—	—	0·00235
Trifluoroperazine	NC	275	18·66	106·250	31·35	18·18	23·89	80·681	37·29	0·00322

* NC, non-competitive; UC, uncompetitive. Values for P_{50} (50 per cent inhibition) are given for an amine concentration of 1 mM. The relationships between a, α, b, β, ΔG_1°, ΔG_2° and P_{50} are given in the text.

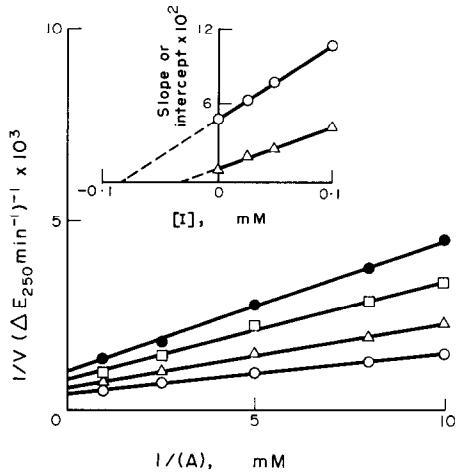


FIG. 1. Double reciprocal plot for nialamide as an inhibitor of the human placental diamine oxidase. (○) *p*-Dimethylaminomethylbenzylamine; (△) *p*-dimethylaminomethylbenzylamine + 0·02 mM nialamide; (□) + 0·05 mM nialamide; (●) + 0·1 mM nialamide. Insert shows replots of intercept (○) and slope (△) as functions of inhibitor concentration ([*I*] mM), giving $K_{i\ slope}$ and $K_{i\ intercept}$ values.

Figure 1 gives double reciprocal plots for nialamide with placental DAO and the insert shows the linear replots of slope and intercept effects.

Figure 2 gives similar kinetic data for pig kidney DAO and nialamide.

Figure 3 illustrates the inhibition of placental DAO by prochlorperazine and the intercept and slope replots are parabolic as shown in the inset. Tangents to the parabolas are also shown replotted as functions of *I*.

In addition to the results in Tables 1 and 2, the following drugs were tested with placental DAO and found to cause no appreciable inhibition at the concentrations

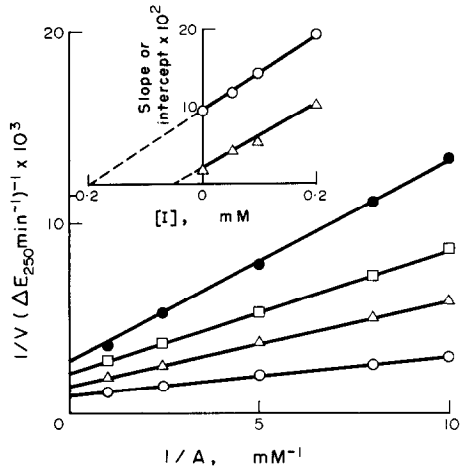


FIG. 2. Double reciprocal plot for nialamide as an inhibitor of the pig kidney diamine oxidase. (○) *p*-Dimethylaminomethylbenzylamine; (△) *p*-dimethylaminomethylbenzylamine + 0·05 mM nialamide; (□) + 0·1 mM nialamide; (●) + 0·2 mM nialamide. Insert shows replots of intercept (○) and slope (△) as functions of inhibitor concentrations ([*I*] mM), giving $K_{i\,slope}$ and $K_{i\,intercept}$ values.

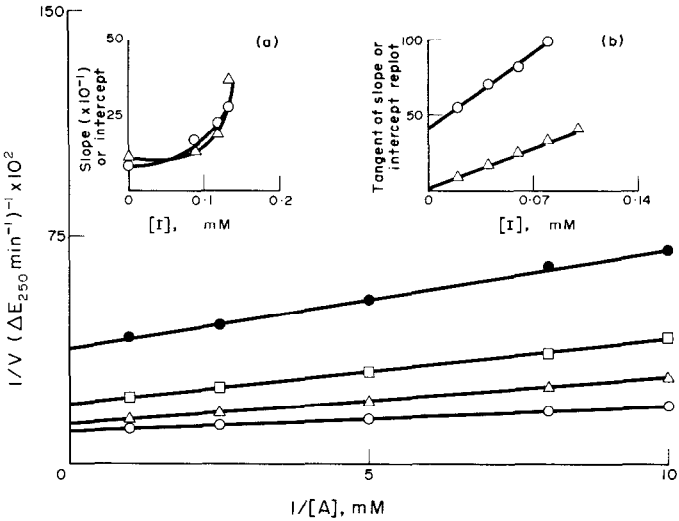


FIG. 3. Double reciprocal plot for prochlorperazine as an inhibitor of human placental diamine oxidase. (○) *p*-Dimethylaminomethylbenzylamine; (△) *p*-dimethylaminomethylbenzylamine + 0·1 mM prochlorperazine; (□) + 0·12 mM prochlorperazine; (●) + 0·13 mM prochlorperazine. Insert (a) shows replots of slope (○) and intercept (△) as functions of inhibitor concentration ([*I*] mM), while insert (b) illustrates a plot of tangents to the parabolas (○, slope; △, intercept) as functions of inhibitor concentration ([*I*] mM).

indicated: ampicillin (1 mM), diazoxide (0·2 mM), ergometrine (0·001 mM), isoprenalin (0·1 mM), lignocaine (1 mM), morphine (1 mM), orciprenalin (0·1 mM), oxytocin (1 unit), papaveretum (approx. 20 mM), phenytoin (0·1 mM) and sodium amytal (1 mM).

DISCUSSION

Hitherto, it has been believed that *bis*-guanidinium compounds were among the best non-competitive inhibitors of diamine oxidase (e.g. for the pentamethylene *bis*-guanidinium species $K_{i\ \text{slope}} = 0{\cdot}00198$ mM, $K_{i\ \text{intercept}} = 0{\cdot}011$ mM with the placental diamine oxidase and $K_{i\ \text{slope}} = 0{\cdot}04$ mM, $K_{i\ \text{intercept}} = 0{\cdot}14$ mM with the pig kidney diamine oxidase). In comparison, phenelzine gives a $K_{i\ \text{slope}}$ of 0·0015 mM with the placental diamine oxidase, and a $K_{i\ \text{slope}}$ of 0·0025 mM and a $K_{i\ \text{intercept}}$ of 0·0025 mM with the pig kidney diamine oxidase. Also, hydralazine ($K_{i\ \text{slope}} = 0{\cdot}000016$ mM, $-\Delta G^{\circ} = 43{\cdot}7$ K J mol^{-1}; $K_{i\ \text{intercept}} = 0{\cdot}00024$ mM, $-\Delta G^{\circ} = 37{\cdot}1$ K J mol^{-1}) was found to be the most potent inhibitor of placental DAO yet discovered.

Acknowledgements—We thank T. Wall & Sons (Meat and Handy Foods) Ltd. for supplying pig kidneys, the Medical Research Council for a grant towards the purchase of a Cary 118C spectrophotometer used in this work, the nursing staff of St. Mary's Hospital, Manchester for assistance in obtaining placentae and Dr. I. V. Scott for advice on drugs used in current obstetric practice.

REFERENCES

1. W. G. BARDSLEY and J. S. ASHFORD, *Biochem. J.* **128,** 253 (1972).
2. M. J. C. CRABBE and W. G. BARDSLEY, *Biochem. J.* **139,** 183 (1974).
3. E. A. ZELLER, in *Metabolic Inhibitors* (Eds. R. M. HOCHSTER and J. H. QUASTEL), Vol. II, p. 53. Academic Press, New York. (1963).

4. W. P. BURKARD, K. F. GEY and A. PLETSCHER, *Biochem. Pharmac.* **11,** 177 (1962).
5. W. G. BARDSLEY and M. J. C. CRABBE, *Biochem. Soc. Trans.* **1,** 494 (1973).
6. W. G. BARDSLEY, M. J. C. CRABBE and J. S. SHINDLER, *Biochem. J.* **131,** 459 (1973).
7. W. G. BARDSLEY, M. J. C. CRABBE and I. V. SCOTT, *Biochem. J.* **139,** 169 (1974).
8. P. A. SHORE and V. H. COHN, *Biochem. Pharmac.* **5,** 91 (1960).