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A BENZYLAMINE OXIDASE DISTINCT FROM MONOAMINE OXIDASE B—WIDESPREAD DISTRIBUTION IN MAN AND RAT

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Abstract—Benzylamine oxidase (BzAO) and monoamine oxidase type B(MAO-B) both selectively catalyse the oxidative deamination of benzylamine (Bz). We define the former as that benzylamine-metabolizing activity insensitive to 4×10^{-4} M deprenyl, a concentration which totally inhibits all forms of MAO. Although both enzymes are widespread in human and rat tissues, their organ distribution differs. Liver and brain show highest MAO-B activity, whilst BzAO activity predominates in aorta and lung. Relatively low BzAO and no MAO-B activity is present in plasma. In the rat, phenylethylamine (PEA) and dopamine (DA) are both substrates for a deprenyl-resistant enzyme with a distribution similar to BzAO, but in man these amines are solely oxidized by MAO. At pH 7.2 the K_m of BzAO for benzylamine is 2.2×10^{-5} M in the rat; μn man, it is 1.1×10^{-4} M. The K_m of MAO-B for benzylamine is 1.0×10^{-4} M in the rat and 5×10^{-5} M in man. Semicarbazide, procarbazine and carbidopa are potent inhibitors of BzAO and inhibit it selectively, leaving MAO substantially unaffected.

Monoamine oxidase (monoamine: O_2 oxidoreductase (deaminating) EC 1.4.3.4.) (MAO) is a mitochondrial flavoprotein enzyme which oxidatively deaminates a wide range of monoamines[1]. Two forms have been distinguished by Johnston[2], although such a classification is at best approximate[3]; type A selectively deaminates 5-hydroxytryptamine (5-HT); type B prefers phenylethylamine (PEA) and benzylamine (Bz) as substrates[4]. Evidence has also accrued that a different enzyme, benzylamine oxidase (BzAO), is able to catalyse the oxidation of Bz[1]. It is not a flavoprotein, but is copper-dependent[1] and may use pyridoxal as cofactor[1]. This enzyme, which has been identified in the blood of many species, has been extensively purified from human plasma and characterized by McEwen[5, 6]. It bears a strong resemblance to a Bz-oxidizing activity distinct from MAO-B, which has been sporadically described in tissues as various as bovine aorta[7], rat heart[8], rabbit lung[9], rat artery[10], and many organs in the pig, particularly in connective tissue[11].

One of us has recently shown[12] that serum BzAO activity is significantly decreased in patients who have sustained severe burns, and in patients with cancer. In an attempt to elucidate the significance of these observations, we decided to embark on a study of BzAO activity and inhibitor sensitivity patterns in human and rat tissues. BzAO can be conveniently distinguished from MAO by selective inhibition with deprenyl, to which MAO-B is particularly sensitive but which inhibits all forms of MAO in higher concentration, leaving BzAO unaffected. Although the Bz-oxidizing MAO-B has aroused greater interest[1, 8–10, 13–17], no fully systematic attempt has so far been made to study the distribution of either enzyme in human or rat tissues.

MATERIALS AND METHODS

Chemicals. Benzylamine hydrochloride methylene[^{14}C] was purchased either from ICN Pharmaceuticals, Inc., Irvine, U.S.A. (sp. act. 12.5 mCi/m-mole), or from Radiochemical Centre Ltd., Amersham, U.K. (sp. act. 56 mCi/m-mole). The radiochemical purity of both was 99 per cent. [^{14}C]Phenylethalamine, sp. act. 50.98 mCi/m-mole, was purchased from New England Nuclear, Boston, U.S.A., and [^{14}C]dopamine, sp. act. 62 mCi/m-mole, from Radiochemical Centre Ltd., Amersham, U.K.

The following compounds were kindly donated: deprenyl by Professor J. Knoll, Budapest, by arrangement with the Chinoin Co., Budapest; procarbazine hydrochloride (Ro 4-6467) and benserazide (Ro 4-4602) by Roche Products Ltd., Welwyn Garden City, U.K.; carbidopa by Merck, Sharp & Dohme Ltd., Hoddesdon, U.K.; and clorgyline by May & Baker Ltd., Dagenham, U.K.

Isoniazid, penicillamine, and β-aminopropionitrile fumarate (BAPN) were purchased from Sigma Chemical Co., St. Louis, U.S.A., pargyline from Abbott Laboratories, North Chicago, U.S.A., and semicarbazide hydrochloride, 99.5 per cent pure (AnalaR grade) from BDH Chemicals, Poole, U.K. Benzylamine, purchased as free base from Sigma London Chemical Co. Ltd., Kingston-upon-Thames, U.K., was converted to its hydrochloride by treatment with hydrochloric acid, and recrystallized. All other reagents were obtained from commercial sources.

Tissues. (a) Rat. Male Wistar rats (200–250 g) were killed by decapitation. Tissues, freed from blood by rinsing in 0.9% saline and drying between layers of filter paper, were coarsely sliced or minced with scalpel or razor blade, quick-frozen in solid CO_2(−80°), and either homogenized immediately with 0.1 M sodium phosphate buffer (pH 7.4),

or stored at −20° for future use. For homogenization, tissues were immersed in liquid nitrogen (−196°) and pulverized by repeated hammer blows in a stainless steel mortar-and-pestle unit. All further steps were carried out at 0°. A 10% (w/v) homogenate in 0.1 M sodium phosphate buffer (pH 7.4) was prepared with ground-glass or Teflon-tipped pestles in 1 ml or 5 ml glass homogenizers, divided into aliquots and stored at −20° until required.

(b) Human tissues were obtained at autopsy from University College Hospital Medical School (kindly arranged by Dr. P. M. Sutton), and treated as described above.

(c) Plasma. Fresh whole blood was collected by venepuncture from normal human donors free of any known disease process, into plastic vials containing lithium heparin (5 units/ml) or K_2-EDTA (1 mg/ml). Within 1–2 hr of collection, it was spun for 10 min at 900 g, divided into aliquots in polypropylene vials and stored at −20° until use. When rats were killed by decapitation, blood was collected into vials containing appropriate amounts of anticoagulant, and dealt with as described above.

Tests were carried out on 4 series of rat tissues and two series of human tissues, unless otherwise stated in the Tables. Replicate assays were carried out (mostly duplicate). Results express the means of the series.

Assay procedures. (a) Radiometric microassay for BzAO in plasma or serum. Potassium phosphate buffer, 0.7 M, pH 7.2 (20 μl), water or inhibitor solution (100 μl), plasma or serum (100 μl) and substrate solution (20 μl) were incubated for 30 min in 75 × 12 mm open polypropylene tubes in a 37° shaking water bath. The final concentration of substrate in the assay tube was 50 μM (rat) or 690 μM (man); that of inhibitors is given in the Tables and graphs. Where inhibitors were used, the mixture was preincubated at room temperature for 20 min before labelled substrate was added. When substrate concentrations higher than 50 μM were used, unlabelled substrate (100 μl in an appropriate concentration) was added immediately before labelled substrate, without preincubation. Blanks were prepared as follows: enzyme and acid blanks, 2 M citric acid (100 μl) was added to the assay mixture before incubation; water blanks were prepared by substituting water for plasma or serum and incubated at 37°. The reaction was stopped by 2 M citric acid (100 μl). Toluene (3 ml) was added to each tube, which was capped, shaken vigorously for 5 min, spun for 5 min at 900 g and placed upright in racks at −20° until the lower phase was frozen solid. The supernatant was then decanted into polypropylene tubes, scintillation fluid (Insta-gel) (4 ml) added and radioactivity measured in a liquid scintillation counter.

Blanks gave radioactivity counts representing ≤0.5 per cent of total substrate activity.

(b) Radiometric microassay for BzAO in tissues. The assay mixture was made up as for plasma, except that the amount of tissue homogenate used instead of plasma was 20 μl for high-activity and 50 μl for low-activity tissue, made up with 80 or 50 μl water. The final concentration of substrate in the assay mixture was 50 μM (rat) or 690 μM (man); that of inhibitors is shown in the Tables and graphs.

(c) K_m. Linearity with time and enzyme concentration. Incubation times and concentrations of substrate and enzyme in the final assay mixture were varied as follows:

(1) K_m: (final concentration)
12.5, 25, 50, 100, 200 and 300 μM [^{14}C]Bz
12,5, 25, 50, 100, 250, 500 and 1000 μM [^{14}C]Bz

(2) Linearity with time and enzyme concentration: incubation times up to 60 min and enzyme concentrations of 5, 10, 20, 30, 40 and 80 μl tissue homogenate, and 5, 10, 20, 40, 100, 200 and 400 μl plasma or serum established the linearity of the reaction under the conditions of the assay described above.

(d) Efficiency of method. After the product of the reaction had been extracted and the supernatant decanted as described, the frozen phase was allowed to thaw, toluene added and the extraction procedure repeated twice. Extraction efficiency was found to be 95 per cent both for plasma and tissues.

(e) McEwen assay of BzAO in plasma or serum. The method of McEwen and Cohen[18], with slight modifications as described previously[12], was used to correlate spectrophotometric and radiometric assays.

(f) Assay of MAO in rat and human tissues, using[^{14}C]PEA and [^{14}C]DA. An extraction procedure similar to that described above was used for DA, and a cation exchange resin method for PEA. Volumes were: buffer 100 μl, inhibitor solution or water 100 μl, enzyme 20 μl and substrate 20 μl; final concentrations of substrate were 312 μM for DA and 27 μM for PEA. For inhibitor studies, mixtures were preincubated at room temperature for 30 min with freshly prepared inhibitor solutions. Blanks were prepared by incubating the buffer–enzyme mixture with 10^{-3} M clorgyline/deprenyl. Otherwise all procedures were as described by Glover *et al.*[19].

(g) The Lowry method[20] was used for determination of total plasma or tissue protein, with bovine serum albumin as standard.

RESULTS

The distribution of Bz and PEA-oxidizing activity in man and rat, considerable in several tissues, is shown in Table 1. It is apparent that more than one enzyme is reponsible for the distribution of activity using the two substrates, as the Bz/PEA ratio is quite dissimilar from tissue to tissue. Liver and brain are the major sources of PEA-oxidizing activity, whereas aorta and lung are most active against Bz. Bz-oxidizing activity in human tissues is many times greater than that of plasma; in the rat, trace activity only is detectable in serum or plasma.

Table 2 shows the effect of deprenyl on deamination of each substrate in different tissues. The two species differ: in the rat, both PEA and Bz oxidation are sensitive to deprenyl, to a varying

Table 1. Specific activities,* using benzylamine (Bz) and phenylethylamine (PEA) as substrates, of rat and human tissues (nmoles/mg protein/30 min)

Tissue	RAT		MAN	
	Bz	PEA	Bz	PEA
Aorta	15.6	7.1	17.5	1.2
Liver	7.4	22.8	14.9	15.2
Lung	6.0	3.5	11.4	1.5
Colon	5.1	2.9	5.8	0.9
Stomach	4.7	3.2	5.7	—
Ileum	4.3	5.3	6.5	0.7
Portal vein	—	—	6.4	—
Duodenum	4.0	3.6	—	—
Pancreas	3.5	21.0	—	—
Tongue	2.4	2.5	3.9	2.5
Adrenal	2.3	1.6	1.9	0.8
Forebrain	2.2	11.6	4.8	7.3
Skin	1.9	—	0.8	—
Oesophagus	1.6	2.8	3.1	1.6
Testis	1.5	2.4	—	—
Brainstem	1.5	8.2	4.1	5.3
Cerebellum	1.4	7.9	4.1	5.0
Diaphragm	1.3	1.3	2.7	—
Spleen	1.1	1.9	1.4	0.5
Parotid	1.1	3.0	—	—
Heart	0.9	3.5	10.3	19.4
Abdominal muscle	0.8	—	4.3	—
Psoas muscle	0.6	0.6	—	—
Kidney	0.6	2.8	10.2	12.1
Serum	0.01	—	0.1	—

* There was close agreement between individual values from human tissues. Rat tissues showed a narrow range of values in some examples, e.g. tongue (mean 2.4, range 2.0–2.8) and diaphragm (mean 1.3, range 1.1–1.4), but wide in others (stomach, mean 4.7, range 1.4–7.5; lung, mean 6.0, range 3.4–9.0). Duplicate assays on the same sample varied by less than 5 per cent.

Table 2. Effect of deprenyl on rat and human tissues, expressed as percentage of enzyme activity inhibited, using benzylamine (Bz) and phenylethylamine (PEA) as substrates

Tissue	RAT		MAN	
	Bz	PEA	Bz	PEA
Pancreas	98	99	—	—
Cerebellum	97	98	97	100
Liver	96	98	96	100
Forebrain	96	99	98	100
Brainstem	89	98	99	100
Kidney	60	91	91	100
Portal vein	—	—	55	—
Parotid	35	97	—	—
Oesophagus	—	—	26	—
Spleen	25	56	32	100
Serum	23	—	0	—
Adrenal	22	63	54	100
Stomach	20	39	15	—
Ileum	20	50	22	100
Skin	16	—	6	—
Tongue	16	52	78	—
Heart	13	90	93	100
Testis	12	62	—	—
Psoas muscle	11	58	—	—
Lung	10	47	12	100
Aorta	9	16	9	100
Duodenum	9	59	—	—
Diaphragm	8	45	65	100
Abdominal muscle	8	—	77	—
Colon	7	43	28	100

degree; in man, however, PEA oxidation is totally inhibited by deprenyl, whereas Bz-oxidizing ability is impaired to an extent varying with the tissue. It must therefore be inferred that in man, all PEA is deaminated by MAO-B, but in rat both PEA and Bz are oxidized by an enzyme or enzymes distinct from MAO-B. The pattern of sensitivity of rat tissues to deprenyl differs according to whether PEA or Bz is employed as substrate; this finding can be explained if the different relative activities of BzAO and MAO-B towards Bz and PEA are taken into account. In rat brain the Bz/PEA activity ratio was 0.18, whereas in aorta it was 2.2.

Table 3 shows the distribution of BzAO (deprenyl-resistant) Bz-oxidizing activity, and Table 4 that of MAO-B (deprenyl-sensitive) in different human and rat tissues. BzAO is most active in aorta, lung and digestive tract. Human plasma has relatively low activity. BzAO is absent from liver and brain, the most active sources of MAO-B.

The isoniazid inhibition pattern of human and rat tissues, employing Bz as substrate, is shown in Table 5. Isoniazid is a selective but weak inhibitor of BzAO and MAO-B in the rat, but in human tissues it is neither potent nor selective (Fig. 1).

In the rat DA-oxidizing activity was also identified, resistant to 4×10^{-4} M deprenyl, a concentration which inhibits both MAO-A and B (Table 6). This activity is likewise highest in aorta and lung and is inhibited by 4×10^{-4} M isoniazid in a manner similar to BzAO, suggesting that this enzyme may contribute to DA oxidation in the rat. In human tissues, however, all DA oxidation is inhibited by 4×10^{-4} M deprenyl, pointing to the converse.

Table 3. Distribution of BzAO activity in human and rat tissues (nmoles/mg protein/30 min). MAO-B was inhibited by 4×10^{-4} M deprenyl. Substrate: Bz, 50 μM (rat); 690 μM (man)

Tissue	Specific activity	
	Rat	Man
Aorta	14.2	15.9
Lung	5.7	10.0
Colon	4.7	4.2
Ileum	4.5	5.1
Stomach	3.9	4.8
Portal vein	—	2.9
Duodenum	3.6	—
Tongue	2.0	0.9
Adrenal	2.0	0.9
Skin	1.6	0.8
Testis	1.3	—
Diaphragm	1.2	0.5
Parotid	0.9	—
Liver	0.9	0.6
Spleen	0.8	1.0
Heart	0.8	0.7
Abdominal muscle	0.8	1.0
Psoas muscle	0.5	—
Kidney	0.2	0.9
Brainstem	0.2	0.04
Cerebellum	0.1	0.1
Forebrain	0.1	0.1
Pancreas	0.1	—
Serum	0.01	0.1

Table 4. Distribution of MAO-B in human and rat tissues (nmoles/mg protein/30 min). Substrate: PEA, 27 μM. For definition of MAO-B activity, see text

Tissue	Specific activity Rat	Man
Liver	22.3	15.2
Pancreas	20.8	—
Forebrain	11.5	7.3
Brainstem	8.0	5.3
Cerebellum	7.7	5.0
Heart	3.2	19.4
Parotid	2.9	—
Ileum	2.7	0.7
Kidney	2.5	12.1
Duodenum	2.1	—
Lung	1.7	1.5
Testis	1.5	—
Stomach	1.3	—
Tongue	1.3	2.5
Colon	1.2	0.9
Aorta	1.1	1.2
Spleen	1.0	0.5
Adrenal	1.0	0.8
Diaphragm	0.7	—
Psoas muscle	0.4	—

BzAO and MAO-B show different K_m values for Bz. In rat lung, BzAO gave a K_m of 2.3×10^{-5} M and in caecum 2.1×10^{-5} M, whereas the K_m for MAO-B in rat liver was 1.0×10^{-4} M. The K_m for Bz in human tissues also distinguishes between MAO-B (5.0×10^{-5} M in brain) and BzAO (1.1–1.2×10^{-4} M for aorta, lung and plasma). This difference results in a different relative distribution of Bz-oxidizing activity in the tissues, depending on substrate concentration used. Table 7 shows that in the rat, liver and brain are relatively more active at higher Bz concentrations, compared with aorta and lung. In addition, substrate inhibition of BzAO appears to supervene with higher substrate concentrations.

Figures 2, 3 and 4 show that carbidopa, procarbazine and semicarbazide are all selective inhibitors of BzAO in human tissues. With each, enzyme activity in tissues such as aorta, lung and colon, rich in BzAO, is selectively inhibited by concentrations which have little effect on brain, kidney and liver, predominantly MAO-B-containing tissues.

Of the other potential inhibitors tested, penicillamine had little effect on human plasma enzyme activity, clorgyline and pargyline none. In human tissues, BAPN proved potent but non-selective. In order of potency, carbidopa and procarbazine were the most effective and selective inhibitors of BzAO, followed by benserazide, semicarbazide and isoniazid.

DISCUSSION

Our studies in man and rat point to a close similarity if not identity of plasma and tissue BzAO in each species. Although it has, in the past, received most attention as a plasma enzyme, we demonstrate that an enzyme of closely similar physicochemical properties is widely distributed in the tissues, and that plasma is, in fact, a relatively poor source of activity. We therefore suggest that the term "plasma (or serum) amine oxidase" be abandoned, and that "benzylamine oxidase", first proposed by Bergeret, Blaschko and Hawes[21], be employed as a provisional name for the enzyme wherever it occurs, until its true function and physiological substrate(s) can be identified.

Table 5. Inhibition by isoniazid (4×10^{-4} M) of enzyme activity in human and rat tissues, using Bz as substrate. Residual activity is expressed as nmoles/mg protein/30 min

Tissue	Rat % inhibition	Rat Residual activity	Man % inhibition	Man Residual activity
Diaphragm	95	0.1	70	0.4
Aorta	93	1.1	79	3.7
Lung	91	0.6	70	3.4
Colon	83	0.9	81	1.1
Abdominal muscle	82	0.1	60	1.7
Psoas muscle	81	0.1	—	—
Tongue	81	0.5	74	1.0
Skin	81	0.4	92	0.1
Ileum	79	0.9	71	1.9
Adrenal	79	0.5	61	0.7
Serum	78	0.002	92	0.01
Duodenum	77	0.9	—	—
Spleen	77	0.2	83	0.2
Stomach	76	1.1	77	1.3
Testis	76	0.3	—	—
Heart	69	0.3	68	4.3
Kidney	55	0.3	47	5.4
Parotid	38	0.7	—	—
Forebrain	31	1.5	57	2.1
Pancreas	29	2.5	—	—
Brainstem	27	1.1	67	1.4
Cerebellum	26	1.0	73	1.1
Liver	19	6.0	21	11.8

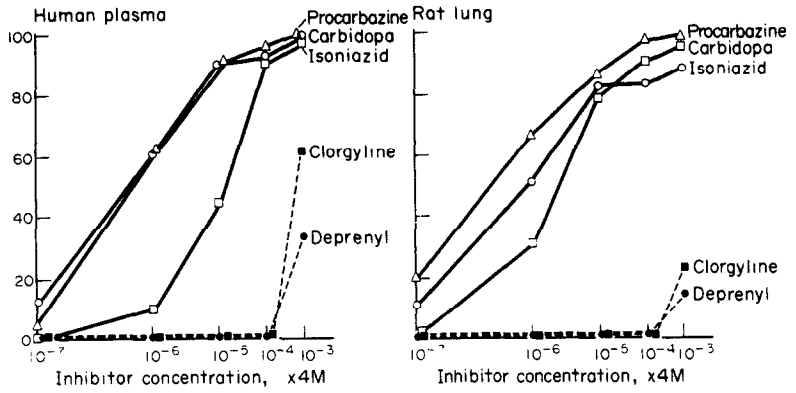

Fig. 1. Effect of selective inhibitors on *in vitro* benzylamine-oxidizing activity in human plasma and rat lung. Each point represents the mean of duplicate determinations at each concentration of inhibitor.

Table 6. Dopamine (DA) oxidizing activity of BzAO in human and rat tissues (nmoles/mg protein/30 min). Substrate: DA, 312 μM. Inhibitor: deprenyl, 4×10^{-4} M

Tissue	Rat			Man	
	Specific activity	Deprenyl % inhibition	Residual activity	Specific activity	Deprenyl % inhibition
Pancreas	72.7	99	0.7	—	—
Liver	64.5	99	0.6	71.3	100
Forebrain	33.4	99	0.3	9.8	100
Heart	31.7	98	0.6	30.3	100
Brainstem	22.6	98	0.4	6.5	100
Cerebellum	17.9	100	0.0	6.4	100
Ileum	14.2	43	8.1	2.2	100
Aorta	12.2	19	9.9	1.6	100
Adrenal	10.1	95	0.6	3.3	100
Colon	8.8	68	2.8	1.8	100
Duodenum	8.6	68	2.8	—	—
Kidney	8.4	96	0.3	36.0	100
Stomach	8.0	69	2.5	—	—
Lung	6.4	88	0.8	6.4	100
Oesophagus	5.9	72	1.6	0.9	100
Tongue	5.4	82	1.0	10.9	100
Testis	5.4	—	—	—	—
Diaphragm	3.9	90	0.4	—	—
Spleen	3.7	96	0.1	0.9	100
Parotid	3.6	—	—	—	—
Psoas muscle	2.2	99	0.02	—	—

BzAO is most highly active in aorta and lung. Recent work on lung and blood vessels of rat [10], rabbit [9, 22] and pig [11, 23] suggests that this semicarbazide-sensitive form of amine oxidase originates in the walls of blood vessels, particularly the endothelial lining. However, these data do not imply that its activity is invariably associated with blood vessels. Buffoni *et al.* [11] found fluorescent antibody cross reaction in connective tissue distinct from blood vessels. They did not identify any activity in liver parenchyma or brain; fluorescence was, however, present in interlobular connective tissue of the liver and in meninges and blood vessels of the brain. These findings agree closely with those of the present study; we were unable to detect BzAO in liver parenchyma or brain, the most active sources of MAO-B, although considerable BzAO activity was present in the portal vein. One fact stands clear. Because plasma BzAO activity is very low, blood contamination [13] can no longer be invoked to account for the high values present in certain tissues.

The inhibition pattern of BzAO with a number of different inhibitors did not appear to vary significantly from tissue to tissue. However, there are pointers in the literature to the existence of isoenzymes of plasma BzAO[24] and multiple forms which differ in their substrate and inhibitor specificities cannot yet be excluded.

Although the distribution patterns of MAO-B and BzAO activities in different tissues are fairly similar in man and rat, the properties of BzAO differ in the two species. In the rat, the K_m for Bz at pH 7.2 is approximately 2.2×10^{-5} M, whereas in man it is about 1.1×10^{-4} M. The rat enzyme also

Table 7. Distribution of Bz-oxidizing activity in rat tissues, employing two different substrate concentrations. Each value represents the mean of 3 series of tissues. Activity expressed as nmoles/mg protein/30 min

Tissue	Benzylamine 50 μM	Benzylamine 690 μM	Ratio
Aorta	15.6	2.2	1:0.1
Liver	7.4	17.6	2.4
Lung	6.0	4.3	0.7
Colon	5.1	2.5	0.5
Stomach	4.7	1.5	0.3
Ileum	4.3	2.3	0.5
Duodenum	4.0	5.2	1.3
Pancreas	3.5	8.4	2.4
Tongue	2.4	1.1	0.4
Forebrain	2.2	7.1	3.3
Oesophagus	1.6	0.5	0.3
Testis	1.5	0.5	0.4
Brainstem	1.5	4.9	3.3
Cerebellum	1.4	2.4	1.8
Diaphragm	1.3	0.5	0.4
Spleen	1.1	0.9	0.8
Parotid	1.1	1.0	0.9
Heart	0.9	1.0	1.1
Psoas muscle	0.6	0.3	0.5
Kidney	0.6	2.2	3.6

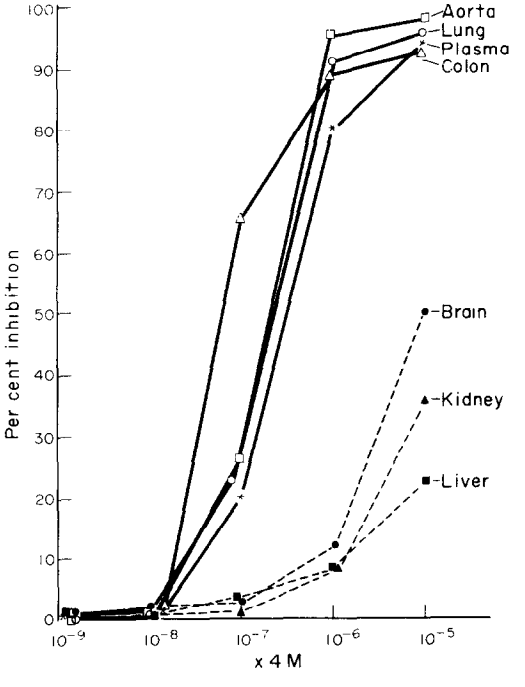

Fig. 2. Selective inhibition of benzylamine oxidation in various human tissues by carbidopa. Each point represents the mean of duplicate determinations at each concentration of inhibitor.

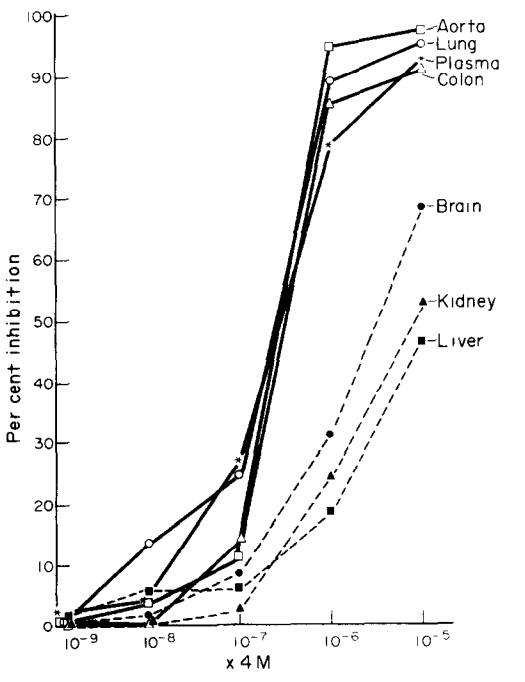

Fig. 3. Selective inhibition of benzylamine oxidation in various human tissues by procarbazine. Each point represents the mean of duplicate determinations at each concentration of inhibitor.

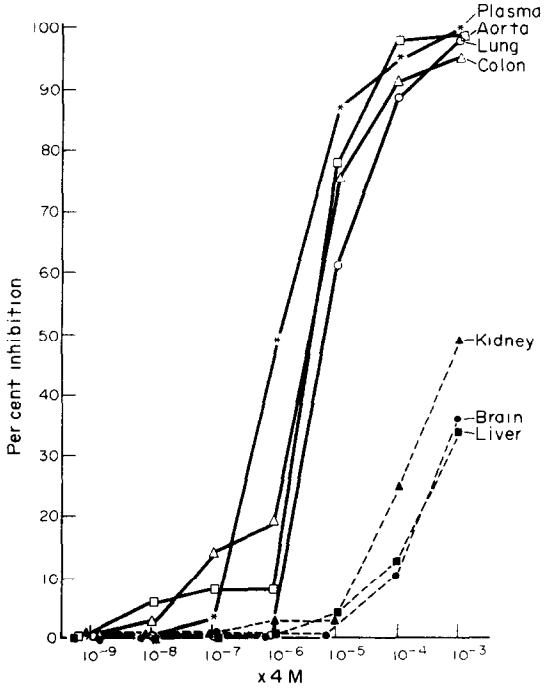

Fig. 4. Selective inhibition of benzylamine oxidation in various human tissues by semicarbazide. Each point represents the mean of duplicate determinations at each concentration of inhibitor.

deaminates PEA and DA, whereas these amines are not substrates of BzAO in man, in whom PEA and DA appear to be oxidized solely by MAO.

The K_m of BzAO for Bz is highly sensitive to changes in pH [6, 25], a property to be taken into account when comparing results from different laboratories. Nevertheless, our results agree well with those of others [8, 25]. Lyles and Callingham [8], distinguishing BzAO from MAO-B in the rat heart, noted K_m values of 10^{-5} M and 5×10^{-4} M respectively. They also observed, and we confirm,

that BzAO is inhibited by high concentrations of substrate, an observation which parallels that of McEwen on rabbit serum enzyme [26].

The function of BzAO is at present unknown. Bz itself has not so far been identified in man or rat, and seems unlikely to be the natural substrate. Lysyl oxidase is known to be present in connective tissue but differs from BzAO both chemically [27] and immunologically [13, 28]. Nevertheless, it is worth noting that two BzAO inhibitors, BAPN and benserazide, are respectively responsible for the connective tissue disease, lathyrism [1] and a condition not unlike it in rats [29].

Semicarbazide was previously known to be a potent selective inhibitor of BzAO [30]. It is of interest that procarbazine and carbidopa have now also been established as potent inhibitors of this enzyme and selectively inhibit it with respect to MAO-B. Carbidopa, a peripheral decarboxylase inhibitor, is used extensively with levodopa for the treatment of Parkinsonism [31], whilst procarbazine is administered for certain types of neoplasm [32]. Both drugs would be inhibitory to BzAO in the dosage regimens employed in clinical practice. Thus, once again, supposedly specific drugs are shown to possess a multiplicity of actions. Careful clinical observation in patients treated with these inhibitory drugs may well shed light on the function or functions of BzAO in man.

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REFERENCES

1. H. Blaschko, *Rev. Physiol. Biochem. Pharmac.* **70**, 83 (1974).
2. J. P. Johnston, *Biochem. Pharmac.* **17**, 1285 (1968).
3. C. J. Fowler, B. A. Callingham, T. J. Mantle and K. F. Tipton, *Biochem. Pharmac.* **27**, 97 (1978).
4. K. F. Tipton, M. D. Houslay and T. J. Mantle, in *Monoamine Oxidase and its Inhibition.* (Eds G. E. W. Wolstenholme and J. Knight), Vol. 39, p. 5. Ciba Foundation Symposium. Elsevier, Excerpta Medica, North-Holland, Amsterdam (1976).
5. C. M. McEwen, *J. biol. Chem.* **240**, 2003 (1965).
6. C. M. McEwen, *J. biol. Chem.* **240**, 2011 (1965).
7. R. B. Rucker and B. L. O'Dell *Biochim. biophys. Acta* **235**, 32 (1971).
8. G. A. Lyles and B. A. Callingham, *J. Pharm. Pharmac.* **27**, 682 (1975).
9. J. A. Roth and C. N. Gillis, *J. Pharmac. exp. Ther.* **194**, 537 (1975).
10. J. F. Coquil, C. Goridis, G. Mack and N. H. Neff, *Br. J. Pharmac.* **48**, 590 (1973).
11. F. Buffoni, L. Della Corte and D. B. Hope, *Proc. R. Soc. Lond. B* **195**, 417 (1977).
12. R. Lewinsohn, *Clin. Chim. Acta* **81**, 247 (1977).
13. K. T. Yasunobu, H. Ishizaki and N. Minamiura, *Mol. Cell. Biochem.* **13**, 3 (1976).
14. G. G. S. Collins and M. Sandler, *Biochem. Pharmac.* **20**, 289 (1971).
15. R. F. Squires, in *Monoamine Oxidases—New Vistas. Advances in Biochemical Psychopharmacology* (Eds E. Costa and M. Sandler), Vol. 5, p. 355. Raven Press, New York (1972).
16. Y. S. Bakhle and M. B. H. Youdim, *Br. J. Pharmac.* **56**, 125 (1976).
17. T. Nagatsu, G. Nakano, K. Mizutani and M. Harada, in *Monoamine Oxidases—New Vistas. Advances in Biochemical Psychopharmacology* (Eds E. Costa and M. Sandler), Vol. 5, p. 25. Raven Press, New York (1972).
18. C. M. McEwen and J. D. Cohen, *J. Lab. clin. Med.* **62**, 766 (1963).
19. V. Glover, M. Sandler, F. Owen and G. J. Riley, *Nature, Lond.* **265**, 80 (1977).
20. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
21. B. Bergeret, H. Blaschko and R. Hawes, *Nature, Lond.* **180**, 1127 (1957).
22. R. B. Rucker and W. Goettlich-Riemann, *Proc. Soc. exp. Biol. Med.* **139**, 286 (1972).
23. F. Buffoni, R. Pirisino and P. Marino, *Agents Actions* **5**, 429 (1975).
24. A. W. M. Lin and D. O. Castell, *Biochem. Med.* **9**, 373 (1974).
25. D. L. Murphy, C. Wright, M. Buchsbaum, A. Nichols, J. L. Costa and R. J. Wyatt, *Biochem. Med.* **16**, 254 (1976).
26. C. M. McEwen, *J. biol. Chem.* **241**, 4544 (1966).
27. R. C. Siegel, R. C. Page and G. R. Martin, *Biochim. biophys. Acta* **222**, 552 (1970).
28. J. J. Shieh, R. Tamaye and K. T. Yasunobu, *Biochim. biophys. Acta* **377**, 229 (1975).
29. E. Theiss and K. Schärer, in *Monoamines Noyaux Gris Centraux et Syndrome de Parkinson.* Bel-Air Symposium IV (Eds J. de Ajuriaguerra and G. Gauthier) p. 497. Georg. Geneva (1970).
30. R. Kapeller-Adler, *Amine Oxidases and Methods for their Study*, p. 133. Wiley-Interscience, New York, London (1970).
31. M. Sandler, in *Catecholamines*, Vol. XXXIII of Handbook of Experimental Pharmacology, p. 845 (Eds H. Blaschko and E. Muscholl) Springer, Berlin (1972).
32. E. Boesen and W. Davis, in *Cytotoxic Drugs in the Treatment of Cancer*, pp. 101, 168–172. Edward Arnold, London (1969).