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Amine oxidase in human blood vessels and non-vascular smooth muscle

RACHEL LEWINSOHN*

Bernhard Baron Memorial Research Laboratories, Queen Charlotte's Maternity Hospital, Goldhawk Road, London W6 0XG, U.K.

The pattern of amine oxidation was studied in human blood vessels at various stages of development, and in tissues with muscular layers made up predominantly or exclusively of smooth muscle. Specific activity of benzylamine oxidase, present in all vascular tissues examined, was higher in vessels than in other tissue; the enzyme, in organs rich in non-vascular smooth muscle, though lower than in blood vessels, was significantly higher than in striated muscle or connective tissue. The localization of benzylamine oxidase activity in smooth muscle, as opposed to connective tissue, may have important physiological implications.

An association between blood vessels and monoamine oxidase (MAO) has long been known (Thompson & Tickner 1951; De la Lande & Waterson 1968; Rucker & O'Dell 1971; Rucker & Goettlich-Riemann 1972; Spector et al 1972; Coquil et al 1973). Some reports (e.g. Coquil et al 1973) suggest that blood vessels may contain, in addition, an amine oxidase different from MAO in substrate specificity and inhibitor sensitivity. The use of diverse assay procedures has precluded unequivocal identification of this activity as the circulating, clorgyline- and deprenyl-resistant, copper-dependent enzyme which preferentially deaminates benzylamine (Bz) and which Bergeret et al (1957) conveniently called benzylamine oxidase (BzAO). It has been claimed (e.g. by Rucker & O'Dell 1971) that this enzyme activity is located in connective tissue†; such claims, however, are not borne out by recent work (see below).

From our earliest observations on amine oxidases in solid tissues of man and rat (Lewinsohn et al 1978), evidence has pointed to blood vessels and richly-vascularized tissues as the main sites of BzAO activity. Subsequent histochemical studies in these laboratories (Ryder et al 1979) showed BzAO to be localized to the tunica media of human blood vessels. Histochemical methods were also employed by Thybusch (1968) and Hervonen & Korkala (1971) respectively in demonstrating the presence of catecholamines and 5-HT in the human foetal carotid body, and MAO activity in the carotid body of rabbit, cat and dog. When I found the highest specific activity of any human tissue for BzAO (but not MAO) in a foetal carotid artery, a closer look at amine oxidase activity in foetal and neonatal carotid arteries seemed indicated.

The localization of BzAO activity in the vascular† tunica media (Ryder et al 1979) raises the question whether a similar activity may be found in smooth muscle other than vascular. To clear up this point, myometrium (rich in smooth muscle) and adjacent endometrium (virtually devoid of smooth muscle cells) (Bradbury 1975) were studied, as well as upper and lower extremities of human oesophagus, which contain, respectively, striated and non-striated muscle only (Warwick & Williams 1980), and ureter and vas deferens, whose walls have intermediary muscular layers consisting solely of non-striated muscle (Warwick & Williams 1980). Except where stated otherwise, endo- and myometrium, and upper and lower thirds of oesophagus, came from the same specimens.

There appears to be no published report on amine oxidase of any kind in any of these tissues, except for MAO in endometrium (Southgate 1972 (review); Mazumder et al 1980) and studies of MAO in vas deferens of species other than man (Jarrott & Iversen 1968; Jarrott 1971; Dial & Clarke 1977, 1978).

The present report describes the patterns of amine oxidase activity in human adult, neonatal and foetal vessels. Eight blood vessels from one individual adult are shown for the patterns of activities of different

* Correspondence and present address: Department of Pharmacology, University of Cambridge, Hills Road, Cambridge CB2 2QD, U.K.

† In the context of this study, 'connective tissue' is defined as 'connective tissue proper' (Bradbury 1975), made up of intercellular matrix, collagen and elastic fibres, fibroblasts and other cellular elements in varying proportion, but few if any smooth muscle cells. 'Vascular tissue' refers to all blood vessels excluding microcirculations (lymphatic vessels have not been examined).

vessels in the same subject. Although they are not vascular tissues, choroid plexus, meninges and Wharton's jelly have been included to permit comparison with values for adjoining tissues and vessels. Values for tissues rich in non-vascular smooth muscle are compared with those for adult blood vessels, and striated muscle. Substrates employed were benzylamine (Bz), 5-hydroxytryptamine (5-HT) and β-phenethylamine (PEA); inhibitors were (−)-deprenyl, selective for MAO-B, and clorgyline, which at 10^{-6} M irreversibly inactivates MAO-A, whilst 10^{-3} M blocks both MAO-A and -B (Lyles & Callingham 1975; Fowler et al 1978; Lyles & Shaffer 1979).

MATERIALS AND METHODS

Sources of all but the following tissues have been given (Lewinsohn et al 1980b; Ryder et al 1980; Lewinsohn & Sandler submitted for publication). The specimens 's' in Table 1 came from an individual (F, 68 years) who died of coronary heart disease. Samples of aorta were taken from the abdominal region below the diaphragm; those of the inferior vena cava, from the region of its junction with the renal vein. Foetal and neonatal carotid arteries were obtained, respectively, from the Royal Marsden Foetal Tissue Bank (by the kindness of Dr. Sylvia Lawler) and from cases of early neonatal death autopsied at Queen Charlotte's Maternity Hospital (by the kindness of Dr Gillian S. Gau and Dr J. Pryse-Davies). The foetal vessels came from two therapeutic abortions at 20 and 17 weeks' gestation, respectively. The neonates' sex was M, F and F, their gestational ages were 33, 40 and 40 weeks, their birth weights 2110, 1800 and 2280 g and crown-rump lengths 32, 34·5 and 32 cm, respectively. Placental vessels, umbilical vessels and Wharton's jelly came from parturients at Queen Charlotte's Maternity Hospital. Oesophageal tissues from two adults and one child (F, 68; M, 63 and F, 3½ years,

Table 1. Distribution of amine oxidase activity in vascular tissues of man. Values express specific activities (nanomol mg^{-1} protein/30 min); s.e. = standard error of the mean. Where n = 2, individual values are shown. s = specimens from same single individual (see text). Tissues: for preparation, see 'Materials and methods'. Final concentrations of substrates and inhibitor used in assay: Bz, 42μM (Tris buffer, pH 9·0); PEA, 150μM (potassium phosphate buffer, pH 7·2); 5-HT, 371μM (potassium phosphate buffer, pH 7·2); (—)-deprenyl, 4×10^{-7} M. Preincubation 20 min at room temperature (20 °C); incubation 30 min at 37°C in shaking water bath. For other details of experimental conditions, see references. Dev. stage = developmental stage; F = foetus; N = neonate; A = adult. D*Bz = deprenyl-sensitive moiety of Bz oxidation; PEA = β-phenethylamine (without inhibitor); 5-HT = 5-hydroxytryptamine (without inhibitor). ND = not done.

Tissue	Dev. stage	n	BzAO Spec. act.	± s.e.	D*Bz Spec. act.	± s.e.	PEA Spec. act.	± s.e.	5-HT Spec. act.	± s.e.
Aorta (*)	F	7	2·9	0·5	2·2	0·4	0·9	0·2	6·8	1·8
Aorta (*)	N	3	8·0	0·8	3·1	0·5	1·2	0·2	5·1	0·5
Aorta (*)	A	4	27·9	1·0	4·4	1·1	1·5	0·1	4·1	0·5
Aorta (**) media	A	s	20·6		4·5		1·2		2·0	
Aorta (**) adv.	A	s	9·9		2·5		0·7		2·7	
Aorta (**) ath. pl.	A	s	2·4		2·1		0·24		0·14	
Hepatic vessels	N	2	4·0, 4·0		7·2, 6·4		3·4, 1·9		15·0, 7·3	
Hepatic vessels	A	s	17·2		15·9		7·0		5·7	
Mesenteric vess.	A	3	31·6	3·2	7·1	2·3	5·5	1·2	5·2	0·7
Mesenteric lymph node vess. (***)	A	s	14·3		2·9		3·0		19·8	
Pulmonary artery	A	s	44·0		5·9		5·0		9·7	
Splenic artery	A	s	31·5		7·9		8·8		21·4	
Inf. vena cava	A	s	24·0		19·6		6·2		7·7	
Coeliac trunk	A	s	29·9		11·6		2·3		6·6	
Cerebral vess. (*)	A	3	47·8	5·9	0		1·9	0·6	8·3	1·2
Choroid plexus	A	s	1·8		33·7		6·8		3·0	
Meninges	F	5	0·5	0·18	0·7	0·09	0		1·5	0·27
Meninges	N	2	5·6, 1·4		1·7, 1·2		0·8, 1·1		3·4, 4·1	
Placental vess. (†)		11	36·0	2·8	3·0	0·6	ND		12·0	2·6
Umbilical vess. (†)		10	28·2	3·4	1·2	0·4	ND		5·7	0·7
Wharton's jelly(†)		1	0·4		0		ND		4·8	

(*) Values from Lewinsohn et al (1980b). (**) Different regions from same specimen; ath. pl. = atheromatous plaque. (***) Vessels surrounding mesenteric lymph node (not lymphatic vessels). (†) Values from Lewinsohn & Sandler (submitted for publication).

Assays done at least in duplicate. Values shown represent means of replicate determinations.

respectively) were obtained at autopsy. Ureters came from autopsies performed on the same child and three male adults aged 79, 85 and 63 years, and the vasa deferentia from the 79- and 63-year-old subjects of this series.

Reagents and sources from which they were obtained have been listed in previous reports (Lewinsohn et al 1978, 1980a, b).

METHODS

Treatment of tissues. Immediately after dissection, the neonatal carotid arteries were freed from loose connective tissue and divided into proximal and distal portions; each portion was split lengthwise into media and adventitia, washed under running cold water to remove all traces of blood, dried between layers of filter paper, minced with scissors, frozen in dry CO_2 and stored at −20 °C until needed. Sections of endometrium and myometrium were taken from fresh surgical specimens subsequently used in histological and histochemical procedures (Ryder et al 1980). Oesophagus, ureter and vas deferens were freed from fat and loose connective tissue on the outside, split open, and the mucosal surfaces scraped and washed thoroughly under running cold water; the remaining steps up to storage at −20 °C were similar to those for the carotid arteries (see above). For treatment of all other tissues, and description of homogenizing procedures, see Lewinsohn et al (1978, 1980b).

Assay. The radiometric microassay procedure employed to determine amine oxidase activity has been described by Lewinsohn et al (1980 a, b). The linearity of the reaction up to 60 min, and with different enzyme concentrations, has been established (Lewinsohn et al 1978). Final concentrations of substrate (shown in the Tables) were 42 or 86 μM for Bz, 150 μM for PEA and 371 μM for 5-HT. With Bz as substrate, assays were carried out in the presence of 0·1 M Tris buffer, pH 9·0; with PEA and 5-HT as substrates, 0·1 M potassium phosphate buffer, pH 7·2, was used. Clorgyline (10^{-7}, 10^{-6} and 10^{-3} M) and (−)-deprenyl (10^{-7} or 10^{-4} M) were employed as 'differentiating inhibitors' (see 'Results', and Tables). For detailed descriptions of all other steps and volumes, see Lewinsohn et al (1978, 1980a, b).

RESULTS

Interpretation of results of assays with the three substrates mentioned above, is based on criteria discussed in detail in previous reports (Lewinsohn et al 1980a, b) which may be summed up as follows:

Benzylamine is a good substrate for both BzAO and MaO B, but a very poor substrate for MAO-A. (−)-Deprenyl 4×10^{-7} M, a concentration selective for MAO-B, was chosen as 'differentiating inhibitor' for most of our work because of the great sensitivity of human foetal tissues towards this inhibitor, and because no difference had been found in Bz deamination of human lung, rich in MAO-A, in the presence of either 10^{-7} or 10^{-4} M deprenyl (Lewinsohn et al 1980a); in a few adult tissues, the higher concentration of deprenyl was employed (Table 3). The reasons for discounting MAO-A activity against Bz as negligible are based on kinetic data and on the absence of any relation between distribution patterns of 5-HT deamination and deprenyl-insensitive Bz activity (Lewinsohn et al 1980a, b). Bz deamination resistant to 10^{-4} or 4×10^{-7} M deprenyl was, therefore, interpreted as BzAO, and Bz activity sensitive to such concentrations of deprenyl (D*Bz) as MAO-B.

5-HT. Further to previous experiments and the points discussed (see Lewinsohn et al 1980a, b) a number of tissues (circle of Willis, aorta, vasa vasorum, renal artery and vein, renal pelvis) assayed with Bz and considered devoid of MAO-B activity because of their insensitivity to 10^{-3} M clorgyline (which inactivates both MAO-A and -B), were also assayed with 5-HT in the presence of 10^{-6} and 10^{-3} M clorgyline. Only the renal vein showed slight residual activity with 10^{-6} M clorgyline, compatible with experimental error; all others were totally inhibited by this concentration, as was of course all 5-HT oxidation in the presence of 10^{-3} M clorgyline. It therefore seems reasonable to conclude that in the experimental conditions of this study, 5-HT was deaminated solely by MAO-A in the tissues examined.

As for PEA, interpretation of results of assays with this substrate, compared with Bz and 5-HT, is discussed fully in Lewinsohn et al (1980a, b).

Blood vessels. In the experimental conditions employed, the highest specific activity of any amine oxidase in adult human blood vessels was clearly that of BzAO (Table 1). However, deamination of 5-HT gave high values in foetal aorta and in the vessels surrounding the adult mesenteric lymph node (not lymphatic vessels), and both MAO-A and -B showed high activity in neonatal hepatic vessels. BzAO activity in adult aortic adventitia was about half that of the tunica media. The atheromatous plaque, however, showed only 12 and 7 per cent, respectively, of BzAO and MAO-A activity in the media. The mesenteric lymph node, from which the surrounding vessels were taken, was completely

calcified, defying all attempts at homogenization. BzAO activity in these vessels was relatively low for adult vascular tissue, but deamination of 5-HT was high, second only to that in the splenic artery of this series. Since the corresponding spleen showed only 5 per cent of the MAO-A activity noted in the splenic artery, the high activity measured in the latter cannot be explained by contamination of the preparation, e.g. by splenic pulp.

The pattern of enzymic activity of the choroid plexus was remarkable for its high D*Bz value, the more so as MAO-A was low, and deamination of Bz in the cerebral vessels of the same individual (sp.act. = 33·1 nanomol mg^{-1} protein/30 min) was insensitive to deprenyl inhibition. Foetal and neonatal meninges gave low values throughout. Wharton's jelly showed minimal activity against Bz, insensitive to deprenyl inhibition, but MAO-A was moderately active and might derive from the fibroblasts in which the tissue abounds (Bradbury 1975).

The high activity described in the introduction has not been found in any of the other carotid arteries studied (Table 2); specific activity of BzAO in foetal and neonatal carotid agreed closely with earlier findings in human aorta at the same developmental stages (Lewinsohn et al 1980b). One foetal carotid (F.1), however, was remarkably active against 5-HT. Activity in proximal and distal portions of neonatal vessels was not significantly different when whole-wall thickness was assayed, but considerable differences were noted between media and adventitia. However, only differences for MAO-B (D*Bz) between media and adventitia were statistically significant ($P < 0.02$); despite the extremely high value found in the proximal adventitia of subject N.1, those for MAO-A were not. This discrepancy probably reflects the high variability of the specimens examined; in specimen N.2 both MAO-B and -A were relatively low.

Non-vascular smooth muscle. The results of this study are summed up in Tables 3 and 4, which show, respectively, the distribution of specific activity of BzAO, MAO-B (D*Bz) and MAO-A, and the pattern of inhibition of Bz and 5-HT oxidation by (−)-deprenyl and clorgyline in the tissues studied. Fig. 1 compares specific activity of BzAO in ureter, oesophagus (upper and lower thirds), and uterus (myometrium and endometrium) with values for adult blood vessels (mean and s.e. calculated from Table 1), and skeletal muscle (Lewinsohn et al 1980b). The slight difference between values for myometrium and endometrium in Fig. 1 and specific

Table 2. Distribution of amine oxidase activity in human foetal and neonatal carotid artery.

	Specific activity							
	BzAO		MAO-B (D*Bz)		MAO-B (PEA)		MAO-A	
Tissue fraction	Proximal	Distal	Proximal	Distal	Proximal	Distal	Proximal	Distal
Whole vessel—F.1	2·2		1·3		5·2		32·1	
Whole vessel—F.2	1·1		2·4		3·5		19·1	
Whole wall—N.1	8·4	11·3	17·7	11·0	8·2	7·3	37·0	31·3
Whole wall—N.2	5·3	5·3	5·5	10·3	4·2	4·8	8·3	15·0
Whole wall—N.3	9·4	7·4	10·6	10·4	5·6	5·9	18·3	20·9
Whole wall—mean	7·9		10·9		6·0		21·8	
s.e. of mean	0·9		1·6		0·6		4·0	
Media—N.1	4·0	13·7	4·0	6·2	3·3	6·1	9·6	33·8
Media—N.2	8·0	5·3	5·5	8·2	3·0	4·0	8·2	10·6
Media—N.3	12·3	5·6	5·3	4·9	3·3	5·0	10·7	12·1
Media—mean	8·1		5·7		4·1		14·2	
s.e. of mean	1·5		0·5		0·5		3·6	
Adventitia—N.1	12·8	8·9	31·4	17·9	13·2	8·4	64·4	28·8
Adventitia—N.2	2·7	5·3	5·4	12·4	5·3	5·6	8·3	19·4
Adventitia—N.3	6·5	9·3	16·0	15·9	8·0	6·8	25·9	29·6
Adventitia—mean	7·6		16·5		7·9		29·2	
s.e. of mean	1·3		3·2		1·0		7·0	

Specific activity expressed as nanomol mg^{-1} protein/30 min. F = foetus; N = neonate. Numbers after initials identify individuals. For experimental and assay conditions, see 'Materials and methods', and Table 1.

Table 3. Specific activity of amine oxidases in human uterus, oesophagus, ureter and vas deferens. Specific activity = nanomol mg^{-1} protein/30 or 60 min.Reaction was linear at all times up to 60 min. BzAO = moiety of Bz oxidation resistant to, D*Bz = moiety of Bz oxidation sensitive to, inhibition by (—)-deprenyl (4×10^{-7} M/uterus, 10^{-4} M/all others). MAO-A = 5-HT oxidation without inhibitor. For interpretation of results, see 'Results'. Final concentrations: Bz = 86μM; 5-HT = 371μM. For details of experimental and assay conditions, see 'Materials and methods'.

Tissue	Enzyme	n	Spec. activity Mean	± s.e.	P
Uterus, myometrium	BzAO	5	7·5	1·7	< 0·001
endometrium	,,	7	0·7	0·27	
Uterus, myometrium	D*Bz	5	9·6	2·54	N.S.
endometrium	,,	7	6·64	0·7	
Uterus, myometrium	MAO-A	5	4·5	1·5	(*)
endometrium	,,	5	33·7	21·3	
Oesophagus, upper	BzAO	3	7·0	1·1	< 0·001
lower	,,	3	23·3	1·0	
Oesophagus, upper	D*Bz	3	3·8	0·7	
lower	,,	3	3·5	1·54	
Oesophagus, upper	MAO-A	3	7·1	1·7	N.S.
lower	,,	3	11·0	5·3	
Ureter	BzAO	4	18·5	0·4	
Ureter	D*Bz	4	1·2	0·25	
Ureter	MAO-A	4	1·5	0·65	
Vas deferens	BzAO	2	14·1, 17·7		
Vas deferens	D*Bz	2	3·4, 5·7		
Vas deferens	MAO-A	2	0·3, 5·8		

(*) 5-HT oxidation in endometrium is subject to considerable variation, depending on phase of menstrual cycle (Ryder et al 1980). Assays on myo- and endometrium, in duplicate; all others, triplicate. Uterus and oesophagus, 30 min incubation, all others 60 min. Values used for calculations were means of replicate determinations.

activity of BzAO shown in Table 3, stems from the fact that for purposes of comparison, tissues from the same individuals (N = 5) were used in the Figure,

Table 4. Sensitivity of Bz and 5-HT deamination in human tissues to inhibition by (—)-deprenyl (4×10^{-7} or 10^{-4} M), and clorgyline (4×10^{-7} M).

Tissue	Substrate/ inhibitor	n	Per cent inhibition mean	± s.e.	P
Uterus, myometrium	Bz/deprenyl	5	53·8	10·2	N.S.
endometrium	,,	7	92·3	2·1	
Uterus, myometrium	Bz/clorgyl.	3	15·0	8·4	N.S.
endometrium	,,	5	17·6	5·2	
Uterus, myometrium	5-HT/depr.	3	54·7	3·7	N.S.
endometrium	,,	5	46·8	11·5	
Uterus, myometrium	5-HT/clorg.	3	69·0	3·0	< 0·01
endometrium	,,	5	90·2	3·65	
Oesophagus, upper	Bz/deprenyl	3	54·7	3·8	
lower	,,	3	14·7	6·2	
Ureter	,,	4	6·5	1·15	
Vas deferens	,,	2	24, 32		

For experimental and assay conditions, see Table 1, and 'Materials and methods'. For concentration of deprenyl employed, see Table 3.

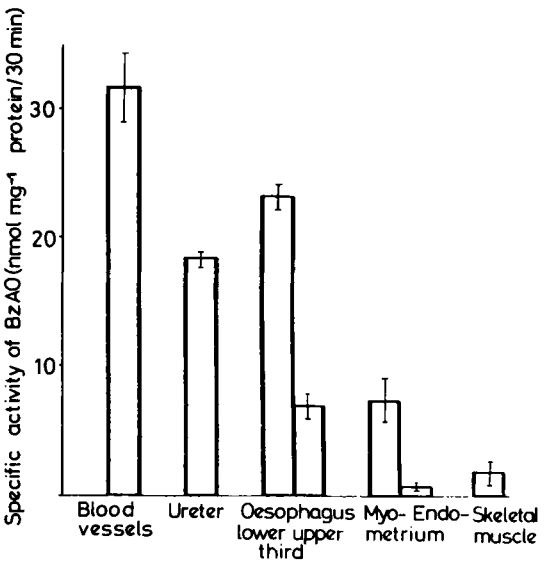

FIG. 1. Specific activity of BzAO in human ureter (n =4), oesophagus (lower and upper extremities) (n = 3), and uterus (myometrium and endometrium) (n = 5) compared with human blood vessels (mean and s.e. calculated from Table 1), and adult skeletal muscle (Lewinsohn et al 1980b). The different regions of oesophagus (lower and upper thirds) were taken from the same organs; similarly, myo- and endometrial tissues came from the same uteri. Vertical bars express s.e. of mean. For experimental and assay conditions, see 'Materials and methods', and Tables 1 and 3.

whereas the means in Table 3 (N = 7) include two endometrial biopsy specimens, where myometrium was not available. Neither ureter nor oesophagus showed age-related differences in amine oxidase activity.

DISCUSSION

The existence of an amine oxidase different from MAO, in vascular tissues of various species, has been reported (De la Lande & Waterson 1968; Coquil et al 1973). Rucker & Goettlich-Riemann (1972) found the properties of rabbit purified aorta amine oxidase to be similar to those of the rabbit plasma enzyme; their description of the latter closely tallies with that of McEwen et al (1966). The experiments of Roth & Venter (1978) on cultured cells strongly suggest the predominance of MAO-B in rabbit aorta endothelium, whilst the immunofluorescence-histochemical studies of Buffoni et al (1977) denote the wide distribution, in porcine tissues, of histaminase, which in this species is identical with the plasma enzyme (Buffoni 1966).

Whatever the explanation of amine oxidase activity

measured in other species, the results here and elsewhere (Lewinsohn et al 1978, 1980b; Ryder et al 1979, 1980; Lewinsohn & Sandler submitted for publication) confirm the localization of BzAO in smooth muscle of human vascular tissue, and, to a lesser degree, in non-vascular tissue rich in smooth muscle. The discrepancy may be largely due to the relative proportions of connective tissue elements and smooth muscle cells in the various organs examined. For example, the low BzAO activity in Wharton's jelly and endometrium, which consist almost exclusively of connective tissue elements, is probably due to vascular fragments in the preparations. Conversely, the major—and in many cases the only—cell type described in the walls of small and medium-calibre mammalian vessels, which make up almost half the cross-sectional area of the entire blood-vascular network in man (Gregg 1966), is the smooth muscle cell (Elias 1961; Somlyo & Somlyo 1968; Cliff 1976). The amount of smooth muscle is inversely proportional to the calibre of the vessel (Elias 1961). Structure varies widely throughout the vascular tree; a universal feature, however, is the presence of one smooth muscle cell layer in the smallest vessels, which is in direct contact with endothelium on the inner (luminal) surface, and with the surrounding connective tissue on the outer (abluminal) side (Elias 1961).

Varied patterns of enzyme activity throughout the vascular tree, such as noted in Tables 1 and 2, may well reflect, amongst other things, the pharmacological heterogeneity of smooth muscle cells in blood vessels (Somlyo et al 1965), differentiated response patterns to vasoactive substances in various regions of the intact circulation (Mellander & Johansson 1968) and the changes which the subendothelial space undergoes with development and ageing (Buck 1963; Cliff 1967, 1970; Gerrity & Cliff 1972). Despite individual differences, BzAO appears to be fairly evenly distributed in the walls of neonatal carotid arteries, but high values for MAO-B (D*Bz) and striking activity of MAO-A suggest the localization of these enzymes, predominantly, in the neonatal adventitia. High specific activity of MAO-A, exceeding both MAO-B and BzAO, in foetal and neonatal carotid arteries may, perhaps, be attributed to a high proportion of fibroblasts, immature smooth muscle and endothelial cells compared with more mature vessels. Such a pattern is suggested by experiments on cultured fibroblasts from human skin, and cultured smooth muscle cells and fibroblasts from young rat aorta (Lewinsohn, unpublished data). High activity of BzAO was found only in the smooth muscle cells, in which Bz oxidation was totally resistant to 4×10^{-7} M deprenyl, whilst the value for MAO-A greatly exceeded that found in fibroblasts. In the latter, Bz oxidation was almost totally sensitive to 4×10^{-7} M deprenyl, denoting the presence of mere traces of BzAO, and moderate activity of MAO-B; 5-HT oxidation, without inhibitor, was high (cf. e.g. Edelstein et al 1978).

As for non-vascular smooth muscle, the results presented here leave little doubt about the localization of BzAO, in the human body, in smooth muscle and not connective tissue. Obviously, part of the BzAO activity in smooth-muscle organs must derive from the walls of blood vessels within them; it is not easy to estimate the proportion of total activity attributable to vascular smooth muscle in any tissue—if, indeed, the enzyme is the same. Further open questions are, whether the smooth muscle enzyme is identical with plasma or serum BzAO, with which it shares many characteristics (Lewinsohn, unpublished data), whether it is the same in different populations of smooth muscle cells in a given organ, and in different tissues, and the function of BzAO in smooth muscle, vascular or non-vascular. Some inferences may perhaps be drawn from clinical observations suggesting that BzAO may be involved in the secretory rather than the vasomotor activity of the cell. There appears to be universal agreement on the production of elastin and collagen by the smooth muscle cell (for review, see Somlyo & Somlyo 1968). Apart from disease, injury and pregnancy, some remodelling of vascular tissue must be going on constantly in the normal individual; the ubiquity and high activity of BzAO in vessels strongly suggest that it may well be a link in the chain of underlying biochemical reactions. Although BzAO is probably not concerned with growth itself (Lewinsohn et al 1980b; Lewinsohn & Sandler submitted for publication), the behaviour of the circulating enzyme in certain pathological states (Lewinsohn 1977) and its strong association in human pregnancy with likely periods of intense vascular development and repair (Lewinsohn & Sandler submitted for publication), suggest that BzAO may participate in angiogenesis or tissue morphogenesis. However, the suggestion that BzAO may be involved in the synthesis of connective tissue fibres does not imply its identity with lysyl oxidase, another copper-dependent enzyme whose role in the maturation of collagen and elastin has been firmly established, but which differs from BzAO in physicochemical and other characteristics (Siegel et al 1970; for review, see Yasunobu et al 1976).

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REFERENCES

Bergeret, B., Blaschko, H., Hawes, R. (1957) Nature (London) 180: 1127–1128

Bradbury, S. (1975) Hewer's Textbook of Histology for Medical Students (revised by S. Bradbury) 9th edn, Heinemann, London, pp 65–81, 118–134, 383–388

Buck, R. C. (1963) in: Sandler, M., Bourne, G. H. (eds) Atherosclerosis and Its Origin. Academic Press, New York pp 16–17

Buffoni, F. (1966) Pharmacol. Rev. 18: 1163–1199

Buffoni, F., Della Corte, L., Hope, D. B. (1977) Proc. R. Soc. London Ser. B 195: 417–423

Cliff, W. J. (1967) Lab. Invest. 17: 599–615

Cliff, W. J. (1970) Exp. Mol. Pathol. 13: 172–189

Cliff, W. J. (1976) Blood Vessels. Cambridge Univ. Press, Cambridge, U.K.

Coquil, J. F., Goridis, C., Mack, G., Neff, N. H. (1973) Br. J. Pharmacol. 48: 590–599

De la Lande, I. S., Waterson, J. G. (1968) Ibid. 34: 8–18

Dial, E. J., Clarke, D. E. (1977) Res. Commun. Chem. Pathol. Pharmacol. 17: 145–156

Dial, E. J., Clarke, D. E. (1978) Biochem. Pharmacol. 27: 2374–2375

Edelstein, S. B., Castiglione, C. M., Breakefield, X. O. (1978) J. Neurochem. 31: 1247–1254

Elias, H. (1961) in: Luisada, A. A. (ed.) Development and Structure of the Cardiovascular System. McGraw-Hill, New York, pp 187–197

Fowler, C. J., Callingham, B. A., Mantle, T. J., Tipton, K. F. (1978) Biochem. Phamacol. 27: 97–101

Gerrity, R. G., Cliff, W. J. (1972) Exp. Mol. Pathol. 16: 382–402

Gregg, D. E. (1966) in: Best, C. H., Taylor, N. B. (eds). The Physiological Basis of Medical Practice. 8th edn, Livingstone, Edinburgh, p. 639

Hervonen, A., Korkala, O. (1971) Experientia 27: 449–450

Jarrott, B. (1971) J. Neurochem. 18: 7–16

Jarrott, B., Iversen, L. L. (1968) Biochem. Pharmacol. 17: 1619–1625

Lewinsohn, R. (1977) Clin. Chim. Acta 81: 247–256

Lewinsohn, R., Böhm, K.-H., Glover, V., Sandler, M. (1978) Biochem. Pharmacol. 27: 1857–1863

Lewinsohn, R., Glover, V., Sandler, M. (1980a) Ibid. 29: 777–781

Lewinsohn, R., Glover, V., Sandler, M. (1980b) Ibid. 29: 1221–1230

Lyles, G. A., Callingham, B. A. (1975) J. Pharm. Pharmacol. 27: 682–691

Lyles, G. A., Shaffer, C. J. (1979) Biochem. Pharmacol. 28: 1099–1106

Mazumder, R. C., Glover, V., Sandler, M. (1980) Ibid. 29: 1857–1859

McEwen, C. M., Cullen, K. T., Sober, A. J. (1966) J. Biol. Chem. 241: 4544–4556

Mellander, S., Johansson, B. (1968) Pharmacol. Rev. 20: 117–196

Roth, J. A., Venter, J. C. (1978) Biochem. Pharmacol. 27: 2371–2373

Rucker, R. B., Goettlich-Riemann, W. (1972) Proc. Soc. Exp. Biol. Med. 139: 286–289

Rucker, R. B., O'Dell, B. L. (1971) Biochim. Biophys. Acta 235: 32–43

Ryder, T. A., MacKenzie, M. L., Pryse-Davies, J., Glover, V., Lewinsohn, R., Sandler, M. (1979) Histochemistry 62: 93–100

Ryder, T. A., MacKenzie, M. L., Lewinsohn, R., Pryse-Davies, J., Sandler, M. (1980) Ibid. 67: 199–204

Siegel, R. C., Page, R. C., Martin, G. R. (1970) Biochim. Biophys. Acta 222: 552–555

Somlyo, A. P., Somlyo, A. V. (1968) Pharmacol. Rev. 20: 197–272

Somlyo, A. V., Sandberg, R. L., Somlyo, A. P. (1965) J. Pharmacol. Exp. Ther. 149: 106–112

Southgate, J. (1972) Adv. Biochem. Psychopharmacol. 5: 263–269

Spector, S., Tarver, J., Berkowitz, B. (1972) Pharmacol. Rev. 24: 191–202

Thompson, R. H. S., Tickner, A. (1951) J. Physiol. (London) 115: 34–40

Thybusch, D. (1968) Acta Histochem. 30: 192–194

Warwick, R., Williams, P. L. (eds) Gray's Anatomy, 36th edn, Longman, Edinburgh (1980) pp 1318, 1402–1403, 1417

Yasunobu, K. T., Ishizaki, H., Minamiura, N. (1976) Mol. Cell. Biochem. 13: 3–29