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MONOAMINE OXIDASE ACTIVITIES OF PORCINE VASCULAR ENDOTHELIAL AND SMOOTH MUSCLE CELLS

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Abstract—Amine uptake by cultured vascular cells was studied under conditions minimizing non-enzymic oxidation. 5-Hydroxytryptamine (5HT) was accumulated only very poorly; detailed kinetic analysis could not be performed, but there was no evidence for a saturable high affinity process. Comparison of β-phenylethylamine (PEA) and 5HT metabolism in intact cells and lysed cells demonstrated that the rates of entry of the amines into cells usually limited their metabolism especially at low (μM) concentrations. Primary cultures of aortic endothelial cells metabolised 5HT and PEA substantially faster than did subcultured endothelium. Subcultured aortic vascular smooth muscle cells and endothelial cells metabolised PEA and 5HT with comparable specific enzyme activities to those found in aortic medial tissue. Inhibition by clorgyline of PEA, 5HT and benzylamine (BZA) metabolism revealed, however, that while aortic tissue possessed monoamine oxidase (MAO) types A and B and a comparable amount of a clorgyline resistant amine oxidase(s) (CRAO), cultured vascular cells possessed MAO-A, but little or no CRAO or MAO-B. Cultured venous endothelium, and smooth muscle from several vascular sites, metabolised PEA and 5HT at similar rates to those found in aortic cells. The studies demonstrate that although cultured porcine endothelial and smooth muscle cells from large blood vessels contain MAO, they do not apparently possess the amine transport process present in the lung. Additionally, conditions of culture can affect both the extent of amine metabolism and the pattern of amine oxidase present.

INTRODUCTION

Circulating vasoactive amines are inactivated mainly by uptake and subsequent metabolism by the cells of the blood vessels. Much of this inactivation takes place in the lung [see e.g. 1, 2], which contains the largest capillary bed in the body, and there is evidence that endothelial cells may be chiefly responsible [3–7]. The relative contributions made by the endothelium and by other cells (in particular, by subjacent vascular smooth muscle cells) are difficult to determine except by isolating the individual cell types and studying them separately. Capillary endothelial cells from the lung have not so far been grown in culture, but both endothelial and smooth muscle cells from larger vessels can be successfully cultured.

There is evidence that such cells share some (though not necessarily all) of the properties of lung cells. For example, cultured endothelial and smooth muscle cells possess monoamine oxidase [8–10]. In addition, an active transport process for 5HT§ similar to that found in the lung has been described in cultured bovine and porcine aortic endothelium [8, 11–13], although more recent work from our laboratory has cast doubt on the existence of this uptake mechanism [14, 15]. Vascular smooth muscle has also been suggested to possess an active uptake mechanism for 5HT [16, 17].

Experiments with cultured cells are complicated by the instability of amines in culture medium but this can be largely overcome by taking stringent precautions to minimise non-enzymic oxidation. In the present studies we found no evidence for a specific, high affinity transport process for 5HT in either cultured porcine aortic endothelial or smooth muscle cells; this suggests either that cells from large blood vessels may not share the same capacity to transport and/or metabolise amines as that possessed by the lung microvasculature, or that these characteristics might be lost when vascular cells are grown in culture. Insufficient radioactivity was retained by the cultured cells to allow a detailed study of uptake kinetics or specificity, so we investigated 5HT and PEA metabolism by intact and lysed cells, to provide an indirect measure of uptake.

We then further characterized the types of MAO activity in cultured porcine endothelium and smooth muscle and compared them with those of freshly

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§ Abbreviations; BZA, benzylamine; CRAO, clorgyline resistant amine oxidase; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid; 5HIAA, 5-hydroxyindoleacetic acid; 5HT, 5-hydroxytryptamine; MAO, monoamine oxidase; PEA, β-phenylethylamine.

isolated aortic tissue. Two forms of MAO have been defined on the basis of their differential sensitivity to inhibition by clorgyline: MAO-A is much more sensitive than is MAO-B [18]. It is generally considered that 5HT is mainly a substrate for MAO-A and PEA is usually a substrate for MAO-B, although this simple classsification may be inadequate [19]. In addition there are amine oxidase activities distinct from MAO; these are resistant to inhibition by clorgyline and deprenyl but are inhibited by semicarbazide, which does not affect MAO. Benzylamine (BZA) is a useful substrate for identifying clorgyline-resistant amine oxidase (CRAO), which is commonly found in plasma, connective tissue and blood vessels [20–23].

MATERIALS AND METHODS

Chemicals. 5-Hydroxy-[G-^{3}H]tryptamine creatinine sulphate (specific activity 500 mCi/mmol) was purchased from the Radiochemical Centre (Amersham, U.K.) and stored undiluted at −196°. 2-Phenyl[1-^{14}C]ethylamine hydrochloride (specific activity 40–60 mCi/mmol) was purchased from the Radiochemical Centre or from New England Nuclear (Dreieich, West Germany) and stored at −20° after dilution to 200 μM in phosphate buffered saline. [7-^{14}C]benzylamine hydrochloride (specific activity 50–60 mCi/mmol) was purchased from the Radiochemical Centre and stored at −20°. Clorgyline and pargyline were gifts from May & Baker (Dagenham, U.K.) and Abbott Laboratories (Chicago, IL) respectively.

Ion-Exchange chromatography. 5HT was separated from its deaminated metabolites using Amberlite CG50 Type II (BDH Chemicals Ltd., Poole, U.K.) which was prepared for use according to the method of Pisano [24] and stored in the acid form. Columns of resin (5 × 10 mm) were rinsed with 5 ml of water followed by 0.5 ml of sample (previously acidified to pH 4 with HCl). Deaminated products were eluted from the column with 2.5 ml water. Radioactivity in subsamples of the starting material and eluate was determined by liquid scintillation counting (1 ml aqueous sample + 7 ml Packard Scintillator 299).

PEA was separated from its deaminated products using Dowex 50W-X8, 200–400 mesh (BDH Chemicals Ltd) which was prepared, stored and used according to Sharman [25]. Columns of resin (5 × 10 mm) were prepared and rinsed with 3 ml 2M HCl, 5 ml water, 3 ml of 1M sodium acetate buffer (pH 6.5) and a further 3 ml of water. Following this, 0.5 ml of sample (previously acidified to pH 4 with HCl) was added and deaminated products were eluted with 2.5 ml water. Radioactivity was determined as above.

Thin layer chromatography. 5HT was separated from its breakdown products by t.l.c. on 0.25 mm silica gel layers containing fluorescent indicator (Polygram; Camlab, Cambridge) using methyl acetate: isopropanol: 25 per cent ammonia (45:35:20 by vol.) as the solvent system [26]. Spots were located under u.v. light, and the plates were cut into 10 mm strips which were transferred to scintillation vials. One millilitre of water was added to each vial, followed by 7 ml of scintillation fluid, and radioactivity determined.

Cell culture. All strains were established from neonatal to 10 day old pigs of the Babraham herd (Large White), and used in primary culture or after 1–10 passages. Endothelial cells were isolated by collagenase treatment of thoracic aorta or superior vena cava [27]; smooth muscle cells were grown from explants of vascular medial tissue [28] or isolated from proteolytic digests using collagenase and elastase [29]. The culture methods for vascular cells have been previously described [30].

Amine metabolism by vascular cells and aortic segments. Confluent layers of cells were grown (*ca.* 10^5 cells per well) in 16 mm dia wells of 24 well Falcon tissue cultures plates (Scientific Supplies Ltd., London, U.K.). The growth medium was removed and the cells were rinsed in phosphate buffered saline [31]. The cells were then incubated with radiolabelled amine (usually at 1 μM or 1 mM final concentration) for 3 hr at 37° in 0.5 ml 20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonate (HEPES)-buffered minimal essential medium [32] (Flow Laboratories, Irvine, Ayrshire, U.K.) pH 7.4 containing 20 μg/ml ascorbic acid and 20 μg/ml EDTA. Control incubations were performed in wells containing no cells. Deaminated products were separated by ion exchange chromatography after acidifying the medium to pH 4 with 0.5 ml of 10 mM HCl. Replicate experiments were performed with lysed cells, by incubating radiolabelled amine with cells as described above but substituting 1 mM potassium phosphate buffer for HEPES-buffered minimal essential medium. Reactions were stopped by adding 0.5 ml of 5 mM HCl.

Amine metabolism was also studied in freshly isolated segments of thoracic aorta from 4–12 day old pigs. Branches were ligated, and 4 cm segments of aorta were perfused at 2.5 ml/min for 15 min at 37° with HEPES-buffered minimal essential medium to ensure that there were no leaks. 2.5 ml of medium containing ascorbate, EDTA and radio-labelled amine was then continuously recirculated at 2.5 ml/min for 30 min at 37°. The medium was then acidified to pH 4 and deaminated products were separated by ion exchange chromatography. Replicate experiments were performed with aortas which had been treated with collagenase [27] to remove the endothelium.

Characterization of amine oxidases. A pooled preparation from neonatal porcine thoracic aortas was used to characterize amine oxidase activity. The tunica intima plus tunica media was stripped from the luminal surface of the aorta immediately after each animal was killed, and stored in 154 mM NaCl at −20°. The combined tissues were homogenized at 4° in 1 mM potassium phosphate buffer (pH 7.8) at a tissue:buffer ratio of 1 g:10 ml, using a tissue disintegrator (Ultra-Turrax, Janke & Kunkel; from Camlab, Cambridge, U.K.) The homogenate was centrifuged (600 *g* for 10 min at 4°), the supernatant was filtered through glass wool, and subsamples were stored at −20°. Protein content was measured by the method of Lowry *et al.* [33]. Amine oxidase activities were measured radiochemically in

subsamples from the low-speed supernatant according to the method of Callingham and Laverty [34]. Briefly, 25 μl supernatant was mixed with 25 μl water and 50 μl radiolabelled amine (usually 2 mM, in 200 mM potassium phosphate buffer pH 7.8; specific activities 0.5–2 μCi/μmol) and incubated under oxygen at 37°. Metabolite production was linear for at least 10 min. The reaction was stopped by plunging tubes into ice-water and adding 10 μl of 3M HCl, and deaminated metabolites were extracted into 0.6 ml of water-saturated toluene : ethyl acetate (1 : 1, v/v). 0.4 ml of the organic layer was mixed with 4.0 ml of Packard Scintillator 299 and radioactivity determined by liquid scintillation counting with automatic quench correction. Control incubations were carried out either without supernatants or with HCl present throughout. For inhibition studies, 25 μl of inhibitor in water was preincubated with 25 μl supernatant at 37° for 20 min before addition of substrate.

Pooled preparations from several different strains of subcultured aortic endothelial or smooth muscle cells were used for similar experiments. Cells were suspended by brief treatment with trypsin and EDTA [30] and stored in 154 mM NaCl at −20°. The combined cells were centrifuged (600 *g* for 3 min) and resuspended at a concentration of 1–2 × 10^7 cells/ml in 1 mM potassium phosphate buffer (pH 7.8) at 4°. The cell suspension was vigorously mixed several times during a 30 min period and subsamples of this lysed cell suspension were then stored at −20°. Protein content and amine oxidase activities were measured as described above. Metabolite production was linear for at least 30 min.

RESULTS

Preliminary experiments on 5HT metabolism

Intact endothelial cells when incubated in HEPES-buffered Dulbecco's medium [35] (used for routine culture of vascular cells) apparently metabolized [^{3}H]5HT. This process was not, however, inhibited by pargyline (50 μM), and control samples incubated without cells showed the same amount of 5HT breakdown (Table 1). Further investigation confirmed that 5HT was rapidly non-enzymically degraded at 37° in Dulbecco's medium. After 30 min incubation only 60 per cent of ^{3}H cochromatographed with authentic 5HT (R_f 0.60) on t.l.c. while up to 25 per cent was found in the area R_f 0–0.35. After 2 hr incubation only 5 per cent was found at R_f 0.55–0.65, and the remaining ^{3}H was distributed throughout the area 0–0.55. 5-Hydroxyindoleacetic acid (5HIAA) had an R_f of *ca.* 0.2 in this system. Thus, breakdown products (some of which had a similar polarity to that of 5HIAA) accumulated rapidly and accounted for the apparent metabolism measured by ion exchange chromatography.

In an attempt to overcome this problem, ascorbic acid and EDTA (known to reduce non-enzymic oxidation of catecholamines [36]) were added. When an experiment similar to that in Table 1 was repeated in Dulbecco's medium in the presence of ascorbate and EDTA, breakdown in 30 min incubations was reduced from 27 per cent above the non-incubated control to 2.5 per cent while accumulation of ^{3}H by the endothelial cells dropped from 35 to <5 pmol/10^6 cells. These results support our earlier conclusion [15] that the apparent amine uptake we observed in preliminary experiments with Dulbecco's medium [14] was due predominantly to accumulation of non-enzymically oxidised products in or on the cells. When experiments were performed using minimal essential medium plus ascorbate and EDTA, MAO inhibitors now inhibited the modest amount of metabolism that could be measured; for example, when intact aortic smooth muscle cells were incubated with 2 μM PEA, replicate cultures metabolized 40 ± 1.6 pmol/10^6 cells/hr in controls and 1.2 ± 0.4 in the presence of 50 μM pargyline (means ± S.E.M., 3 determinations).

Metabolism of PEA and 5HT by cultured vascular cells

It was not practicable to undertake experiments on individual cell strains with more than ~10^5 cells for each experimental point, and incubation times of at least 3 hr were necessary to detect metabolism of 5HT or PEA by these relatively small numbers of cells. Consequently, it was necessary to investigate amine stability during these prolonged incubations before any detailed study of cellular metabolism of amines could be performed. Table 2 shows the non-enzymic oxidation of PEA and 5HT in various media and the effects of ascorbate and EDTA. Amine oxidation was unacceptably high in Dulbecco's medium even in the presence of ascorbate

Table 1. Degradation of 5-hydroxy [G-^{3}H] tryptamine in cultures of aortic endothelial cells and in culture medium alone

Incubation time (min)	Per cent degradation		
	Cells alone	Cells + 50 μM pargyline	No cells
0	6.2 ± 0.3	5.9 ± 0.3	5.6 ± 0.2
5	7.6 ± 0.1	8.1 ± 0.4	
10	10.6 ± 0.6	11.4 ± 0.5	
20	22.4 ± 1.3	25.9 ± 0.8	21.4 ± 0.8

Cells (6 × 10^4) were incubated with 2 μM substrate in 0.2 ml Dulbecco's medium for up to 20 min at 37°. The cells were preincubated with or without pargyline for 30 min. 5-Hydroxy[G-^{3}H]tryptamine was separated from ^{3}H-labelled oxidised products by ion exchange chromatography (see Methods). Values are means ± S.E.M. of four determinations.

Table 2. Stability of 5-hydroxy[G-^{3}H]tryptamine and 2-phenyl[1-^{14}C]ethylamine

Incubation medium	Per cent oxidation	
	5-hydroxytryptamine	Phenylethylamine
Dulbecco's medium alone	73.0 ± 7.0	11.9 ± 1.0
Dulbecco's medium + ascorbate and EDTA	33.0 ± 6.2	9.9 ± 0.1
Minimal essential medium alone	32.0 ± 4.0	1.0 ± 0.1
Minimal essential medium + ascorbate and EDTA	5.3 ± 0.6	0.60 ± 0.10
1 mM potassium phosphate buffer alone	8.0 ± 0.9	0.70 ± 0.09
1 mM potassium phosphate buffer + ascorbate and EDTA	7.3 ± 0.8	0.60 ± 0.15
Non-incubated controls	7.2 ± 1.6	0.80 ± 0.08

Amines (1 μM) were incubated in 0.5 ml, with or without the addition of 20 μg/ml ascorbic acid and 20 μg/ml EDTA, for 3 hr at 37°. Radiolabelled non-enzymic oxidation products were separated by ion exchange chromatography (see Methods). Higher concentrations of ascorbic acid (up to 200 μg/ml) or the presence of fetal calf serum (up to 20 per cent v/v) did not alter the results, nor did the use of 1 mM amine concentrations. Values are means ± S.E.M. of 4 observations.

plus EDTA, and minimal essential medium (containing ascorbate and EDTA) was therefore routinely used for experiments with intact cultured cells; 1 mM phospate buffer was used for experiments with lysed cells.

Amine metabolism was measured at low (1 μM) or at saturating (1 mM) substrate concentrations, but it should be emphasized that because of the prolonged incubation times in these experiments the values obtained are unlikely to represent initial rates, and may be used only for comparisons. The results for endothelial cells are summarised in Table 3. At a concentration of 1 μM, metabolism of both substrate by lysed cells was 2–3 fold greater than by intact cells. At 1 mM, this was still true for 5HT but not for PEA, implying that PEA diffuses well enough into endothelial cells at this concentration to saturate the monoamine oxidase activity, whereas 5HT does not. Freshly isolated aortic cells, whether lysed or intact, metabolised both amines at a faster rate than did subcultured cells; additionally, at 1 μM concentrations both substrates were metabolised at similar rates by freshly-isolated cells, in contrast to subcultured cells (where PEA was a preferred substrate).

Experiments were also performed using subcultured smooth muscle cells from 4 vascular sites, and the results are summarised in Table 4. Like endothelial cells, lysed arterial smooth muscle cells metabolised 2–3 fold more PEA at 1 μM than did intact cells, but similar amounts at 1 mM. Smooth muscle cells from any vascular site, like subcultured endothelium, showed a higher rate of metabolism of 1 μM PEA than of 1 μM 5HT. Cells grown from the 3 arterial sites showed similar capacities for amine metabolism, but smooth muscle cells growth from the vena cava metabolised both substrates (at 1 μM concentration) to a greater extent than did arterial cells; this difference was more marked than the difference found between aortic and venous endothelium (Table 3). It is perhaps worth emphasizing that during these experiments no detectable amine or metabolite was accumulated either by endothelium or by smooth muscle cells in culture.

Metabolism of PEA and 5HT by perfused aortas

Segments of aorta perfused with 1 μM PEA or 5HT metabolised 700 ± 60 and 42 ± 8 pmol/segment/hr; if the aorta was treated with collagenase to remove endothelial cells before perfusion the values obtained were 620 ± 40 and 38 ±

Table 3. Metabolism of β-phenylethylamine and 5-hydroxytryptamine by cultured endothelial cells

Substrate	Intact cells			Lysed cells		
	Fresh aortic	Subcultured aortic	Subcultured vena caval	Fresh aortic	Subcultured aortic	Subcultured vena caval
Phenylethylamine 1 μM	145 ± 20	33 ± 7	52 ± 10	340 ± 60	92 ± 15	105 ± 6
5-Hydroxytryptamine 1 μM	124 ± 20	9.5 ± 2.4	15 ± 2	376 ± 90	24 ±4	29 ± 3
Phenylethylamine 1 mM		4.3 ± 0.5	6.9 ± 2.1		5.5 ± 1.1	4.8 ± 1.5
5-Hydroxytryptamine 1 mM		3.4 ± 0.8	4.8 ± 0.7		12.7 ± 0.7	10.6 ± 2.1

Values are means ± S.E.M. of 3–7 replicate observations, expressed as pmol/10^6 cells/hr for 1 μM substrate and nmol/10^6 cells/hr for 1 mM substrate.

Table 4. Metabolism of β-phenylethylamine and 5-hydroxytryptamine by cultured vascular smooth muscle cells

Substrate	Intact cells				Lysed cells			
	Aorta	Carotid artery	Iliac artery	Vena Cava	Aorta	Carotid artery	Iliac artery	Vena Cava
Phenylethylamine 1 μM	37 ± 14	48 ± 5	39 ± 4	101 ± 25	120 ± 7	109 ± 6	97 ± 4	165 ± 15
5-Hydroxytryptamine 1 μM	12 ± 1	14 ± 3	14 ± 5	40 ± 18	12 ± 1	15 ± 3	15 ± 2	80 ± 10
Phenylethylamine 1 mM	6.0 ± 1.6	7.0 ± 3.0	5.0 ± 1.0	7.0 ± 3.0	5.0 ± 1.9	12.0 ± 3.0	8.0 ± 2.0	8.0 ± 4.0
5-Hydroxytryptamine 1 mM	8.0 ±2.0	8.0 ± 2.0	15.0 ± 6.0	9.0 ± 3.0	12.0 ± 5.0	11.0 ± 3.0	12.0 ± 4.0	12.0 ± 5.0

Values are means ± S.E.M. of 3–7 replicate observations, expressed as pmol/10^6 cells/hr for 1 μM substrate, and nmol/10^6 cells/hr for 1 mM substrate.

4 pmol/segment/hr respectively (means ± S.E.M., 3 determinations). Less than 1 per cent of the total radioactivity was retained by the tissue in either case.

Characterisation of amine oxidase activities in porcine aortic tissue and cultured cells

We pooled several strains of cultured cells and several segments of vascular tissue to provide sufficient material to study the inhibition by clorgyline of initial rates of amine oxidation (see Methods for details), and we also examined BZA metabolism, because clorgyline-resistant BZA oxidase had previously been found in porcine aorta [37].

At 1 mM substrate concentrations, similar amounts of PEA, 5HT and BZA were metabolised by porcine aortic medial preparations, in which the cell type is almost entirely smooth muscle (see Table 5). PEA and 5HT were metabolised at comparable specific activities by lysates of cultured smooth muscle or endothelial cells; however, little BZA metabolism was detectable in cultured cells (<12 per cent of the rate of metabolism of PEA or 5HT). At 1 μM substrate concentrations, unlike 1 mM, both aortic tissue and smooth muscle cells metabolised PEA to a much greater extent than 5HT, in agreement with the previous experiments summarised in Table 4.

To characterise the enzymes responsible for this metabolism, replicate experiments were performed in the presence of increasing concentrations of clorgyline. Figure 1 shows the effect of clorgyline on the metabolism of 1 mM amines by aortic tissue. 5HT gave a simple sigmoid curve with an IC_{50} of about

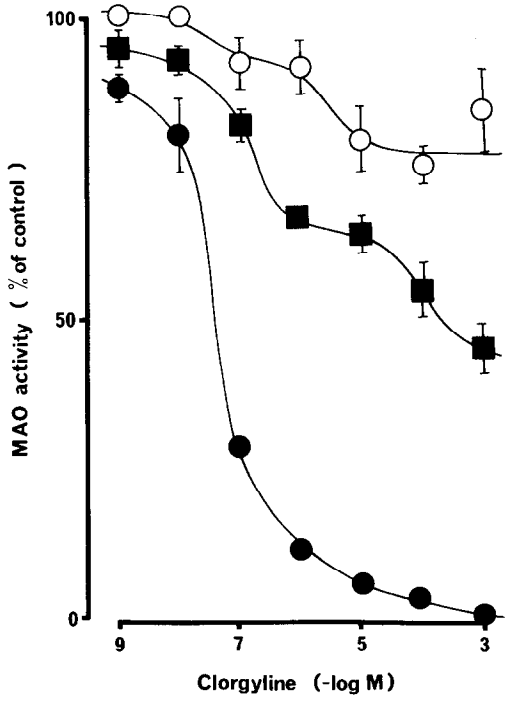

Fig. 1. Effect of clorgyline on amine oxidase activity in porcine aortic tissue. Ten minute incubations; substrate concentration 1 mM. Points are means of 5 (5HT, ●; and PEA, ■) or 6 (BZA, ○) observations, ± S.E. where this exceeds the symbol size.

Table 5. Amine oxidase activity in low-speed supernatant from homogenised porcine aorta and in lysates of cultured aortic endothelial and smooth muscle cells

Substrate	Amine oxidase activity (nmol/mg protein/hr)		
	Aortic tissue	Smooth muscle cells	Endothelial cells
1 μM phenylethylamine	4.6	10.9	
1 μM 5-hydroxytryptamine	0.25	1.0	
1 mM phenylethylamine	44.2 ± 7.4	41.6 ± 6.8	39.6 ± 9.2
1 mM 5-hydroxytryptamine	32.4 ± 1.6	34.8 ± 7.8	32.4 ± 2.6
1 mM benzylamine	39.0 ± 9.4	< 5	< 5

Samples were incubated for 5–30 min with substrates. Values are means ± S.E.M. (5 or 6 observations), or means of triplicates (duplicates for benzylamine in cultured cells).

50 nM clorgyline, indicating that this substrate was metabolised almost exclusively by MAO-A. PEA showed a double sigmoid curve with a plateau, and in addition there was substantial activity (~40 per cent of the total) resistant to inhibition by 1 mM clorgyline, suggesting that PEA was metabolised by MAO types A and B and also by CRAO. BZA oxidation was ~80 per cent resistant to inhibition by 1 mM clorgyline. In further experiments we investigated the effects of 1 mM clorgyline and 1 mM semicarbazide on the metabolism of 1 mM PEA or BZA. The rates of oxidation (means of 3 determinations) expressed as a percentage of the rates in the absence of inhibitors were as follows: PEA plus clorgyline 42; PEA plus semicarbazide 65; PEA plus both inhibitors 5; BZA plus clorgyline 72; BZA plus semicarbazide 23; BZA plus both inhibitors 4.

The metabolism of PEA and 5HT by lysates of cultured smooth muscle and endothelial cells in the presence of clorgyline was analysed similarly, and examples of the inhibition profiles are shown in Fig. 2. With smooth muscle cells (Fig. 2a) both 1 mM 5HT and PEA gave simple sigmoid curves with similar IC_{50} values of 50 nM clorgyline, suggesting that the activity was almost entirely MAO-A, but using 10 μM or 100 μM PEA (data not shown) a small amount of MAO-B activity (10–20 per cent of the total) could be detected. There was no residual activity against 5HT, but perhaps a little clorgyline-resistant oxidation (<10 per cent) of PEA. In agreement with this, we have detected a small amount of oxidation of 10 μM BZA by some lysed smooth muscle cell preparations; this was inhibited by semicarbazide. With endothelial cells (Fig. 2b) the deamination of 5HT and PEA was apparently due entirely to MAO-A; no MAO-B or CRAO activity was found using either PEA or 5HT as substrates.

DISCUSSION

5HT and PEA are rapidly deaminated during passage through microvascular beds by the action of MAO following cellular uptake of the amines, apparently by the capillary endothelial cells [see 1, 2, 38]. Detailed studies of amine uptake and metabolism by lung capillary endothelium (uncomplicated by the presence of other tissues) would require the

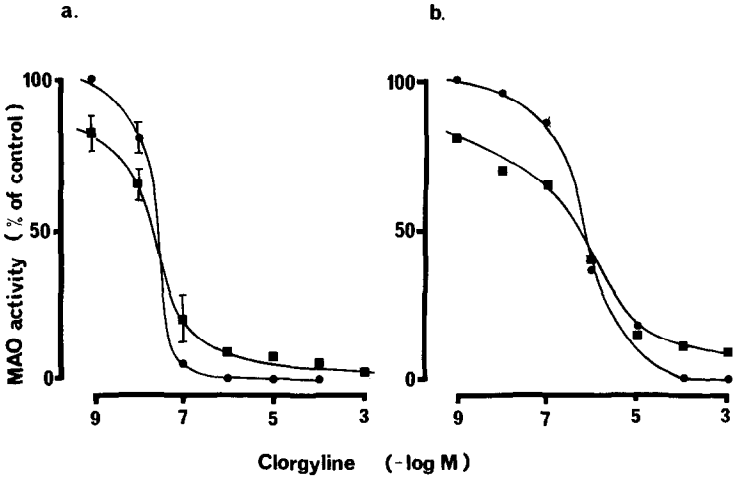

Fig. 2. Effect of clorgyline on amine oxidase activity in lysed cultures of porcine aortic cells. Thirty minute incubations; substrate concentration 1 mM. 5HT, ●; PEA, ■. (a) Smooth muscle cells; points are means of 5–8 observations ± S.E. where this exceeds the symbol size. (b) Endothelial cells; points are means of 3 observations.

isolation and culture of these cells, and techniques for this are not yet routinely available. Several workers have used cultures of endothelium from large blood vessels for such studies, in the knowledge that these cells exhibit at least some of the characteristics of capillary endothelium [30, 39, 40]. Shepro *et al.* [11] suggested that cultured bovine aortic endothelium possessed a transport system for 5HT similar to that described in the lung; Small *et al.* obtained similar results and also demonstrated the presence of MAO in these cells [8]. Initial results from our laboratory and others indicated that cultured porcine endothelium also actively transported 5HT [12, 13]. Our subsequent experimental results, however, do not support this conclusion; in conventional tissue culture medium 5HT is unstable and readily undergoes non-enzymic oxidation, and when precautions are taken to minimise this breakdown, uptake of 5HT is very slow and cannot be attributed to a saturable uptake process of the type found in porcine lung slices [12]. It appears, therefore, that previous studies may have been estimating the accumulation of auto-oxidation products of 5HT. We were also unable to detect any active uptake process for 5HT in cultures of vascular smooth muscle cells. Further work is needed to establish whether active transport of 5HT occurs only in certain regions of the vascular tree, and whether vascular cells capable of transporting 5HT retain this property in culture.

Although there was insufficient accumulation of radioactivity by the cultured cells in our experiments to investigate in detail the kinetics and specificity (if any) of amine uptake, it was clear that metabolism of 5HT or PEA followed by release of the deaminated product occurred. In general, amine metabolism was lower in intact than in lysed cells, suggesting that metabolism was limited by rate of entry into the cells, especially at low (1 μM) concentrations. An alternative explanation would be that MAO was activated under the different conditions used in the experiments with lysed cells, but this seems unlikely because the changes observed after cell lysis varied with cell type and substrate (see Tables 3 and 4).

Metabolism of PEA or 5HT was higher in freshly isolated aortic endothelium than in subcultured cells, suggesting that the levels of MAO may decrease with age in culture, as has been noted *in vivo* [41]; it is also possible that the isolation procedure temporarily increases MAO activity, as occurs with prostaglandin synthesis [42]. Experiments with perfused aortic segments support the idea that MAO activity was elevated by isolating endothelial cells, because the small difference between the rates of 5HT metabolism in perfused aortas with or without endothelial cells present was more consistent with the rate of metabolism in endothelial subcultures than in primary cultures.

Porcine aortic tissue contained MAO-A, MAO-B and CRAO; 5HT was almost exclusively metabolised by MAO-A, BZA mainly by CRAO and PEA by all three enzymes. This ability of pig aortic CRAO to metabolise PEA is similar to that of the rat, although in man this amine is not a substrate for CRAO [22]. The finding that 5HT was solely a substrate for MAO-A is of interest, for although this is generally true, 5HT is a substrate for MAO-B in several other porcine tissues, where type B predominates [43–45]. These three enzymic activities presumably reflect the properties of the smooth muscle cells, because endothelial cells comprise at most about 5 per cent of the cells present, the contribution of MAO activity from noradrenergic nerve endings is likely to be insignificant because of their relative scarcity [46], and the CRAO activity in this tissue is unlikely to be derived from plasma because neonatal pig plasma contains little or none [47, 48].

Subcultured smooth muscle cells metabolised PEA and 5HT with comparable specific activities to those found in the parent aortic tissue, but BZA was metabolised much less well. These cells were proliferating rapidly in culture, and forming little extracellular connective tissue. In view of the postulated roles of CRAO in connective tissue synthesis [20] it would be of interest to examine the enzymic activity in smooth muscle cells cultured under conditions favouring the deposition of an extracellular matrix. A further difference from aortic tissue was revealed by the use of clorgyline; smooth muscle cells in culture exhibited predominantly MAO-A activity, which metabolised both PEA and 5HT. PEA, especially at high concentrations, has been shown to be a substrate for both MAO-A and MAO-B in other tissues [49, 50]. Subcultured aortic endothelial cells apparently metabolised PEA and 5HT exclusively by MAO-A; we could not detect any CRAO or MAO-B activity.

Our results demonstrate that the proportions of MAO (A and B) and CRAO can change dramatically on culturing vascular endothelium and smooth muscle cells; thus, the levels and types of this activity in cultured cells do not always reflect the activity within the parent tissue. Additionally, cultured porcine aortic endothelium does not transport 5HT by a process similar to that apparently present in porcine lung capillary endothelium. It will therefore be valuable to study amine uptake by capillary endothelial cells in culture, for which the techniques are now becoming available [51], although the fact that amine oxidase activity can change in cultured cells must be taken into account in such studies.

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