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Predominance of the B form of monoamine oxidase in cultured vascular intimal endothelial cells

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Until recently the role attributed to endothelial cells has been that of a rather passive lining of blood vessels with little or no metabolic activity of physiological significance. This concept has changed gradually over the past 10 years due, in part, to the discovery that many biochemical and physiological events and systems are associated with endothelial cells from the intima of blood vessels. These findings include the demonstration of contractile elements [1], cell to cell communication [2], fibrinolytic activity [3], angiotensin conversion [4] and numerous drug and neurotransmitter receptors as well as cyclic nucleotide responses in intimal endothelial cells [5]. It has also been demonstrated that lung vasculature, including human lung, can alter the concentration of blood born vasoactive hormones as they transverse the pulmonary circulation [6–10]. Several recent reviews [11–15] have expounded upon the presumed role of the pulmonary vasculature in regulating the systemic circulating levels of a variety of vasoactive substances including angiotensin I and II, bradykinin, prostaglandins and biogenic monoamines. The principle site in lung responsible for these important processes is presumed to be the vascular intimal endothelium [11–15].

Pulmonary disposition of the biogenic vasoactive amines, norepinephrine and 5-hydroxytryptamine, has been extensively studied in a number of laboratories [6–15]. These reports have indicated that both norepinephrine and 5-hydroxytryptamine are actively transported into lung vasculature and subsequently degraded by the enzymes monoamine oxidase (MAO) and/or catechol-*O*-methyl transferase. The rapid appearance of metabolites of norepinephrine and 5-hydroxytryptamine in effluents from lungs perfused *in vitro* suggests that pulmonary amine uptake and degradation occur at or near the vascular luminal surface, presumably the endothelial cell [11–15]. Three functionally distinct forms of amine oxidase exist in the intact perfused lung [16]. Substrate and inhibitor studies indicate that two of the

Table 1. Extent of rabbit aorta intimal endothelial cell deamination of various biogenic amines*

Amine	M	Product (nmoles/mg protein ± S. E.)	Per cent metabolism
Phenylethylamine	8×10^{-6}	47.0 ± 3.6	66.7
Tyramine	1×10^{-4}	63.3 ± 1.6	7.0
5-Hydroxytryptamine	1×10^{-4}	15.7 ± 1.4	1.6
Dopamine	1×10^{-4}	10.1 ± 0.8	1.0
Norepinephrine	1×10^{-4}	5.1 ± 1.1	0.5

* Reaction mixtures containing ^{14}C-labeled biogenic amine and endothelial cell homogenate were incubated for 1 hr at 37° in a total volume of 0.4 ml of 0.067 M potassium phosphate buffer, pH 7.4. Reactions were terminated with the addition of 50 μl of 0.4 M HCl and a 200-μl aliquot of the resulting mixture was chromatographed over a Bio-Rex 70 cation exchange resin column to separate the deaminated products from the starting amine substrate.

MAO subtypes resemble the A and B forms of the mitochondrial oxidase [17] and the third type has properties and characteristics of the "plasma" amine oxidase [18, 19].

Although there is considerable information in the literature to suggest that endothelial cells are the primary site in blood vessels responsible for amine removal and inactivation, direct evidence for this has been lacking. To circumvent problems related to perfusing intact tissues and of indirectly measuring amine disposition in endothelial cells, we have examined amine metabolism in cells cultured from the intimal endothelium of the rabbit aorta [5]. These cultured cells have been reported to retain a morphological and biochemical resemblance to the intact intimal endothelium from which they were derived [5].

For this study, a continuous line of endothelial cells was maintained in culture as previously described [5] in F-12 nutrient medium (GIBCO) in an atmosphere of 5% CO_2. Contact inhibited cells from 100 mm dishes (Falcon) were harvested by scraping with a rubber policeman into phosphate buffered saline. Resulting cell suspensions were precipitated by centrifugation at 10,000 *g* for 15 min. The pellets were gently resuspended in 0.1 M potassium phosphate buffer, pH 7.4, and recentrifuged at 12,000 *g* for an additional 15 min. The cells were resuspended by homogenization in a glass tissue grinder in buffer and MAO activity was assayed essentially as described by Roth *et al.* [20] for cultured human fibroblasts. The concentrations of the amines used were at or near their reported K_m values for MAO [21, 22].

The ability of intimal endothelial cells to deaminate a variety of biogenic amines was examined and results are listed in Table 1. Of the amines tested, phenylethylamine is most rapidly degraded by crude homogenates of these cells and is followed in decreasing order of activity by tyramine, 5-hydroxytryptamine, dopamine and norepinephrine.

As 5-hydroxytryptamine is preferentially deaminated by the A form of MAO and phenylethylamine by the B form [17], the above data suggest that both forms of the mitochondrial oxidase are present to some extent in the endothelial cells. To further verify this, the effect of a selective, type A MAO inhibitor, clorgyline [23], was examined against the deamination of tyramine (a substrate for both the A and B forms of MAO). The clorgyline inhibition experiments (Fig. 1) do not display a typical biphasic plot as observed for other systems [23], but indicate that tyramine in endothelial cell homogenates is metabolized principally by the B form of MAO. The small decrease in tyramine activity produced at 10^{-11} to 10^{-8} M clorgyline (Fig. 1) is interpreted as being due to the inhibition of the A form of MAO by this agent. It is important to note that the data in Fig. 1 represents the

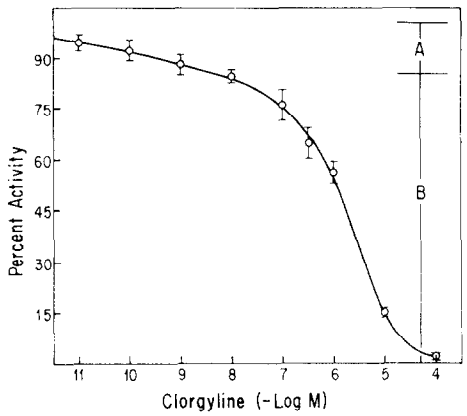

Fig. 1. Effect of clorgyline on tyramine deamination by homogenates of cultured rabbit endothelium. Endothelial cell homogenates (0.043 to 0.16 mg protein) were preincubated for 12 min at 37° in the presence of varying concentrations of clorgyline prior to the addition of [^{14}C]tyramine (final concentration 7.5×10^{-5} M in 0.4 ml of 0.067 M potassium phosphate buffer, pH 7.4). Reactions were incubated for an additional 60 min at which time 0.2-ml aliquots were removed and chromatographed on a Bio-Rex 70 cation exchange resin column. These columns were washed with 2.8 ml water, and 10 ml Aquasol was added to the total effluent. Each point represents the mean ± S. E. of four separate determinations with each performed in duplicate.

mean values of four separate experiments. When each individual experiment was plotted in a similar manner, a more typical biphasic plot was observed for clorgyline inhibition of tyramine. These data strongly suggest that the "predominant" form of monoamine oxidase in the intima is the B form of the oxidase. Therefore, it became important to clearly demonstrate that deamination of 5-hydroxytryptamine (Table 1) is indeed catalyzed by the A form of the enzyme in our experiments.

To this end the effect of harmaline, another type A MAO selective inhibitor [17] was tested against 5-hydroxytryptamine and phenylethylamine deamination. Results summarized in Fig. 2 indicate that 5-hydroxytryptamine deamination was inhibited 65 and 90 per cent by 0.1 and 0.5 μM harmaline, respectively, whereas phenylethylamine deamination was unaffected at the higher harmaline concentration. These data are consistent with the concept that both the A and B forms of the mitochondrial oxidase do exist in rabbit aorta endothelium. No "plasma" amine oxidase activity could be detected as the "plasma"

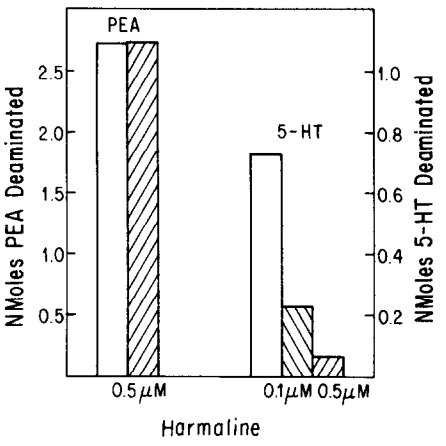

Fig. 2. Effect of harmaline on 5-hydroxytryptamine (5-HT) and phenylethylamine (PEA) deamination by homogenates of cultured rabbit endothelium. Endothelial cell homogenates in a total volume of 0.4 ml of 0.067 M potassium phosphate buffer, pH 7.4, were incubated with either 7.6 μM [^{14}C]phenylethylamine or 0.10 mM [^{14}C]5-hydroxytryptamine in the absence and presence of harmaline, as indicated. Enzyme activity is expressed as the average nmoles of deaminated product formed/60 min of three separate experiments each performed in duplicate. Deaminated product was assayed as described in the legends of Fig. 1 and Table 1.

amine oxidase inhibitor semicarbazide had no effect on the deamination of tyramine by endothelial cell homogenates.

An important aspect of endothelial cell amine disposition in culture would be the demonstration of a correlation with data from studies *in vivo*. In this regard, the substrate specificity and relative activities of the various substrates for cultured rabbit aorta endothelial MAO used herein are remarkably similar to those found with the perfused rabbit lung [24]. The per cent deaminated product appearing in effluents from lungs perfused with phenylethylamine (94 per cent), tyramine (42 per cent), 5-hydroxytryptamine (27 per cent), dopamine (19 per cent) and norepinephrine (7 per cent) followed the same order as that appearing in Table 1. This similarity suggests that rabbit pulmonary endothelial cells may contain a similar proportion of the A and B forms of MAO as that of rabbit aorta endothelium, and that the pulmonary endothelium is indeed the principle site of amine metabolism in lung.

This study demonstrates that intimal endothelial cells can selectively inactivate circulating vasoactive monoamines based on their substrate affinity for either of two forms of the mitochondrial oxidase. Unlike other cultured cells examined to date [20, 25, 26] in which the A form of MAO predominates, endothelium contains mainly the B species of the oxidase. Because the B type of MAO predominates in these cells, substrates such as 5-hydroxytryptamine and norepinephrine which are preferentially deaminated by the A form may be more likely to escape inactivation within the circulation.

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