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MONOAMINE OXIDASE A AND B: A USEFUL CONCEPT?

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The use of a number of inhibitors of monoamine oxidase (monoamine:O_2 oxidoreductase, EC 1.4.3.4) has shown that many mammalian tissues contain two major forms of the enzymic activity that differ in their substrate specificities and sensitivities to inhibitors. Although several pieces of evidence indicate that these two forms may not represent two distinct enzymes but result from a single enzyme species existing in different membrane-bound environments [see eg., 1, 2], it has frequently been assumed that their specificities will be broadly similar in a variety of organs and tissues and also in different animal species. If such an assumption were true it should be possible to extrapolate results found in any convenient laboratory animal to the situation in man. It would seem however, that this general assumption may be an oversimplification. This review is therefore an attempt to assess the usefulness of the concept of the existence of two functionally distinct forms of the enzyme, without exploring the possible nature of any such forms, since this aspect has recently been reviewed in detail elsewhere [3].

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In 1968 Johnston [4] showed that the irreversible inhibitor clorgyline was able to inhibit the activity of rat brain monoamine oxidase, with serotonin as substrate, at considerably lower concentrations than were required to inhibit the activity towards benzylamine. When tyramine was used as the substrate about half of the activity was inhibited at low concentrations of the inhibitor, whereas the remainder was only inhibited with considerably higher concentrations. This gave rise to a biphasic "dose–response" curve such as that shown in Fig. 1. Johnston interpreted these results as indicating the presence of two enzyme species; one, which he termed the A-form, sensitive to inhibition by clorgyline and capable of deaminating serotonin and tyramine and another, the B-form, which was relatively insensitive to inhibition by clorgyline and active towards benzylamine and tyramine [see also ref. 5]. On the other hand deprenil [6] has been shown to inhibit irreversibly the activity towards benzylamine at much lower concentrations than those needed to inhibit the activity towards serotonin. Several other irreversible inhibitors of monoamine oxidase have been reported to have a selective effect on one or other of the two forms of the enzyme.

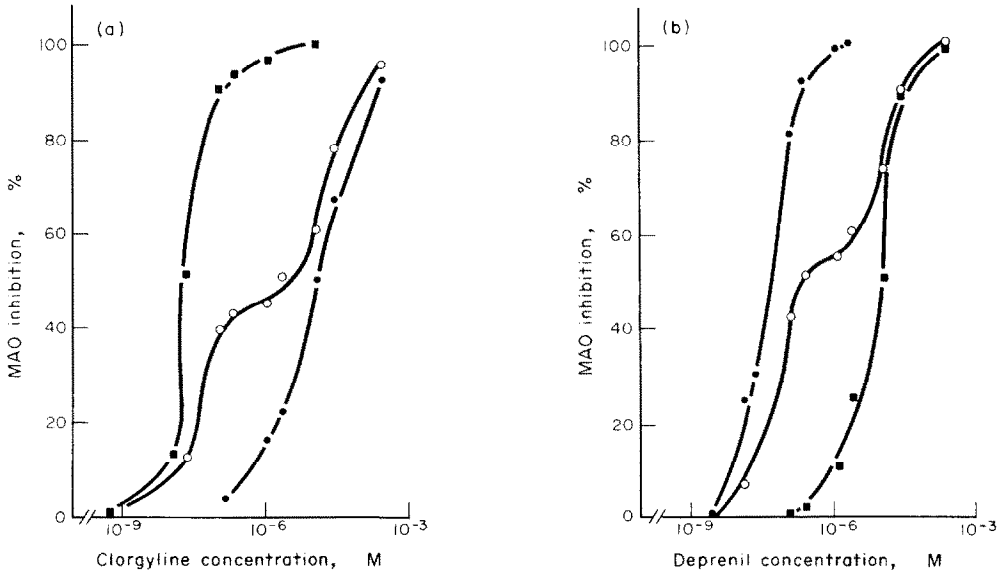

Fig. 1. Clorgyline (a) and Deprenil (b) inhibition of tyramine (○), serotonin (■) and benzylamine (●) deamination catalysed by rat liver mitochondrial monoamine oxidase. It should be emphasised that the experimentally determined pI_{50} values are dependent upon the protein concentration [53, 56] and the ionic strength of the buffer medium [56].

Table 1. Some selective inhibitors of monoamine oxidase

Selective for A-form		Selective for B-form	
Reversible:		*Reversible*:	
Amphetamine	[7]	Imipramine	[15]
Harmine	[8]	Amitriptyline	[16]
Harmaline	[9]		
Mexiletine [1-methyl-2-(2,6-xylyloxy)-ethylamine]	[10]		
1-Methyl-tryptamine	[7, 12]		
Benzylcyanide	[11]		
4-Cyanophenol	[11]		
Irreversible:		*Irreversible*:	
Clorgyline [*N*-Methyl-*N*-propargyl-3-(2,4-dichlorophenoxy)-propylamine]	[4, 5]	Deprenil [phenyl-isopropyl-methyl-propinylamine]	[6]
Lilly 51641 [*N*-[2-(*o*-chlorophenoxy)-ethyl]-cyclopropylamine]	[13]	Pargyline [*N*-methyl-*N*-benzyl-propynylamine]	[17]
PCO [5-phenyl-3-(*N*-cyclopropyl)-ethylamine-1,2,4-oxadiazole]	[14]		

In addition a number of reversible inhibitors are also selective; with K_i values towards substrates for one form of the enzyme that are lower than the K_i values towards substrates for the other. Examples of both types of inhibitor are given in Table 1.

Selective inhibitors have been used to determine the specificity of the two forms in more detail. In studies of the enzyme from rat liver and brain, tyramine, dopamine and kynuramine have been shown to be substrates for both forms of the enzyme [5, 11, 14, 18], adrenaline [11] and noradrenaline [11, 19] have been shown to be substrates for the A-form, and benzylamine and 2-phenylethylamine have been shown to be substrates for the B-form [11, 20]. In studies of the properties of the enzyme from other organs and other species the assumption has often been made that enzyme preparations from these sources will behave in a similar manner to those from rat liver and brain, only differing in the proportions of the two enzyme forms. Thus inhibition of the activity towards tyramine or kynuramine by clorgyline and deprenil has been used to assess the relative proportions of the two forms of the enzyme present by estimating the height of the plateau in the "dose–response" curve (see Fig. 1). In addition these observations have sometimes been supplemented by data from experiments with an A-form substrate such as serotonin and a B-form substrate such as benzylamine or 2-phenylethylamine. Studies of this type show that the apparent proportions of the two forms vary widely between the various organs of the rat [21], and between different animal species [5, 18]. It has also been shown that the B-form of the enzyme, which comprises about half of the total monoamine oxidase in the mature rat liver and brain, is absent from the foetal rat brain 6 days before birth [22], and that it is absent from cultured neuroblastoma and glioma cells [23] and from some rat hepatomas [24].

In an extensive survey of the distribution of the two enzyme forms in 8 mammalian species, Squires [18] noted several discrepancies from the simple binary classification of the enzyme forms. Clorgyline and harmine were found to act as selective inhibitors of the activity of the enzyme from several organs of the rabbit, whereas deprenil and pargyline showed no selectivity with these enzyme preparations. He also noted that several tissues in which the selective inhibitors had failed to reveal any A-form were nevertheless able to oxidise the A-form substrates, serotonin and noradrenaline. Squires did not attempt to discriminate between the possibilities that the oxidation of these substrates was due to the presence of undetected amounts of the A-form or that the B-form was capable of oxidising serotonin and noradrenaline in these particular tissues. In the case of the enzyme from the pig brain, which has been reported to be composed of the B-form only [5, 18], it has been suggested that the activity towards serotonin is due to the presence of small amounts of the A-form [25]. However, with the enzyme from ox liver, studies with harmine [8], clorgyline and deprenil [26] are consistent with serotonin being a substrate for the B-form of the enzyme.

Recently several reports have appeared that suggest that the binary classification into A- and B-forms with defined specificities may not be universally applicable. Studies with clorgyline have shown that benzylamine is a substrate for both forms of the enzyme in the rat heart whereas tyramine is a substrate for the A-form only [27]. Inhibition of the enzyme in the ox heart with both clorgyline and deprenil has shown that serotonin is a substrate for both forms [28]. Biphasic inhibition curves have also been found for the oxidation of 2-phenylethylamine by the enzyme from mouse [29] and ox brain [26], chicken liver [30] and chick heart [31], which would indicate that this amine is a substrate for both forms of the enzyme in these tissues. A recent study of the enzyme from ox liver [26] has shown that, although the oxidation of serotonin, tyramine and 2-phenylethylamine is relatively sensitive to inhibition by deprenil and insensitive to inhibition by clorgyline, the inhibition curves are not consistent with these substrates all being oxidised by the same form of the enzyme. These and several other observations agree with the suggestion of Squires [18], on the strength of thermal inactivation experiments, that both forms of the enzyme are heterogeneous. For example tranylcypromine has been shown to act as a selective inhibitor of the

Table 2. Species and organ differences in the sensitivities of monoamine oxidase activities towards clorgyline

Substrate	Enzyme form responsible for oxidation					
	A-form only		B-form only		Both forms	
Tyramine	Rat heart	[27, 36]	Ox liver	[5, 26]	Rat brain	[4, 11]
			Rabbit heart	[37]	Rat liver	[5, 11]
			Rabbit lung	[38]	Rat vas deferens	[40]
			Cat liver	[5]	Rat sketal muscle	[41]
			Dog liver	[5]	Rat uterus	[42]
			Moorhen heart	[39]	Human brain	[5, 43]
			Moorhen liver	[39]	Human heart	[44]
			Human platelet	[33]	Human liver	[5]
					Rabbit brain	[5]
					Ox brain	[5, 26]
					Chick heart	[45, 46]
					Chick liver	[45]
					Mouse heart	[37]
Serotonin	Rat brain	[4]				
	Rat heart	[27, 36, 37]				
	Rat liver	[5, 11]	Ox liver	[5, 26]	Ox heart	[28]
	Human brain	[5]	Moorhen heart	[39]	Chick liver	[31]
	Human heart	[44]	Moorhen liver	[39]		
	Human liver	[5]	Human platelet	[33]		
	Chick heart	[46]				
2-Phenylethylamine	Rat heart	[37]	Ox liver	[26]	Mouse brain	[29]
			Rat liver	[11]	Ox brain	[26]
			Rat brain	[20]	Chicken liver (adult)	[30]
			Human heart	[44]	Chick heart	[31]
			Chick liver	[31]	Moorhen heart	[39]
			Moorhen liver	[39]		
			Human platelet	[33]		
Benzylamine			Rat brain	[5]		
			Rat liver	[5, 11]		
			Human brain	[43]	Rat heart	[27, 36]
			Human heart	[44]		
			Rabbit heart	[37]		
			Ox liver	[27]		
			Chick liver	[45]		
			Human platelet	[33]		

monoamine oxidase activity in human blood platelets towards tryptamine and 2-phenylethylamine [32] despite the fact that studies with clorgyline and deprenil have shown this source to contain the B-form of the enzyme alone [33]. Differential inhibition of the activity of rat liver enzyme towards benzylamine and 2-phenylethylamine by Tris buffer [34] and the kinetics of inhibition of the enzyme from this source by irreversible inhibitors [14, 35], have also suggested that both the A- and B-forms of the enzyme are heterogeneous.

The literature on the selective inhibition of monoamine oxidase in different organs and species is large and sometimes contradictory. Table 2 lists a selection of the results obtained with the more commonly used substrates, which gives a fair idea of the current state of confusion.

In the light of the results shown in Table 2 and the others discussed above, we feel that the simple classification into A- and B-forms can be misleading. As the substrate specificities and inhibitor sensitivities of the enzyme from many different sources become more fully defined, more and more deviations from the simple binary classification become apparent; a situation not unlike that seen with the adrenoceptor [see 47]. Since the results discussed earlier suggest that the A- and B-forms may represent families of enzyme species, a result consistent with those showing the sensitivity of the enzyme to its lipid environment [1], any attempt to treat the enzyme as being composed of two major forms is likely to be an over-simplification. Perhaps it would be possible to define the enzyme forms in terms of their sensitivity to inhibitors, but even here results obtained with clorgyline and deprenil are not always the same [18], and thus any classification would need to rest solely on the response to one accepted standard inhibitor. Clorgyline, as originally suggested by Johnston [4], would appear to be the most suitable from this point of view, since it has been found to be selective in its effect in cases when deprenil was not [18]. It may be that even this classification is not good enough in view of the observation that, in some tissues of the pig, inhibition with clorgyline gave single sigmoid "dose–response" curves, when the use of harmine and pargyline has shown the presence of both forms of the enzyme [48]. In addition, any classification based on the sensitivity to clorgyline, must recognise the fact that the substrate specificities determined for the enzymes from rat liver and brain, are not universally applicable and thus a range of substrates must be used. From what is known at present, it appears that

serotonin, tyramine, benzylamine and 2-phenylethylamine might be adequate in conjunction with clorgyline sensitivity, to establish a crude classification of the enzyme into A- and B-forms. Nevertheless, as further anomalies come to light, it may be necessary to increase the range of these "standard" substrates. In this context it is unfortunate that difficulties with the commonly used assay methods [see 49] have restricted the use of adrenaline and noradrenaline as substrates in such studies.

Whatever the use of the binary classification, it is clear that attempts to extrapolate from the situation in any one organ or species to that in another could be extremely misleading. A thorough comparison between the characteristics of the enzyme in laboratory animals and man must be made before any particular species is selected as the appropriate experimental model for man.

A final note of caution is appropriate concerning the use of selective inhibitors in *in vivo* studies. Although a number of reports have indicated that clorgyline [50–52], deprenil [52], Lilly 51641 [9, 51] and PCO [53] do act as selective inhibitors *in vivo*, their selectivity may not be so clearly defined as that seen *in vitro* [51], and it may only be short-lived [53, 54]. This loss of selectivity may in part, be due to the nature of the localisation of the enzyme within the target organ and the elevated amine concentrations, resulting from the inhibition of one enzyme form, acting as reversible inhibitors of the other form [55]. Another possibility is, however, the *in vivo* metabolism of the inhibitor to produce compounds with decreased selectivity [53]. Since the selectivity of inhibitors is very sensitive to substitutions in the benzene ring, the microsomal hydroxylase system may function to render such inhibitors non-selective [53]. Further studies on the metabolism of selective inhibitors would be of great value in assessing the significance of such effects. Whatever the cause of this loss of selectivity, it is important to discover if it is a general phenomenon with selective inhibitors. If this were the case their clinical use would be restricted by the same factors that apply to the classical monoamine oxidase inhibitors. At present it seems advisable to find out if treatment with selective inhibitors is in any way an improvement in effectiveness, or in freedom from side effects.

The considerations discussed here indicate that, although the classification into A- and B-forms represented a major advance when it was first introduced by Johnston some years ago, uncritical use of this classification to extend conclusions obtained from one species to another is unwarranted.

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