Biochemical Pharmacology, Vol. 23, pp. 47–56. Pergamon Press, 1974. Printed in Great Britain.

SUBSTRATE SPECIFICITY OF CAERULOPLASMIN. PHENYLALKYLAMINE SUBSTRATES

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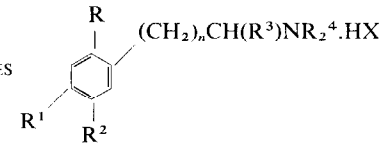
(*Received* 16 *March* 1973; *accepted* 25 *June* 1973)

Abstract—Several phenylalkylamines have been examined as substrates for the copper-containing oxidase caeruloplasmin and it has been shown that the compounds most readily oxidized by this enzyme are those which contain a 3,4-dioxygenation pattern in the aromatic ring. Of these two oxygen atoms, one must be present as a free hydroxy group; the other may be either a hydroxy or methoxy group. A primary amino group may be substituted for one of these oxygen functions without loss of substrate activity. Monophenols are poor substrates for caeruloplasmin. Although the alkylamine side chain is essential for high substrate activity the distance between the basic group of the side chain and the aromatic nucleus is not critical, the higher homologues of dopamine being very good substrates for this enzyme. Monosubstitution at the α-carbon atom of the side chain has relatively little effect on substrate activity but disubstitution at this point markedly reduces substrate activity.

IN A PREVIOUS paper[1] the effects of certain hallucinogenic drugs on the oxidation of noradrenaline, dopamine and 5-hydroxytryptamine by the enzyme caeruloplasmin were described. Although centrally active glycollates[2] and amphetamines[3] had no effect on the caeruloplasmin-catalysed oxidation of these biogenic amines it was shown that lysergic acid *N,N*-diethylamide (LSD) inhibited the oxidation of 5-hydroxytryptamine but enhanced the oxidation of noradrenaline and dopamine. It was additionally shown that tranquillizers, including some phenothiazines, enhanced the oxidation of all three substrates whereas tricyclic antidepressants inhibited the enzymic oxidation of all three substrates. These observations suggested[1,4] that caeruloplasmin, or an enzyme with similar properties, might be intimately concerned with the maintenance of normal function in certain areas of the central nervous system and that interference with this enzyme might result in the appearance of abnormal mental states. It therefore seemed desirable on the one hand to investigate caeruloplasmin in more detail, particularly with reference to its substrate specificity, and on the other hand to determine whether the central nervous system contained an enzyme which was identical with, or had properties similar to, caeruloplasmin. The present paper reports the results of some studies on the substrate specificity of caeruloplasmin.

Known substrates for caeruloplasmin[5–8] include noradrenaline, dopamine, adrenaline, 5-hydroxytryptamine and substituted *p*-phenylenediamines in addition to ascorbate and Fe^{2+}. It can be seen therefore that caeruloplasmin acts on a variety of substrates of different chemical types. From the point of view of this investigation it seemed likely that the substrates of greatest potential significance were those related to the natural transmitter substances noradrenaline, dopamine and 5-hydroxytryptamine. The present report deals with substituted phenylalkylamines as substrates for caeruloplasmin; a subsequent paper will deal with indolic substrates.

Table 1. Analytical data on some phenylalkylamines



R	R^1	R^2	R^3	R^4	n	m.p.°	X	Molecular formula	Analysis					
									Calculated			Found		
									C	H	N	C	H	N
H	OH	CH_3O	H	H	1	204	Cl	$C_9H_{14}ClNO_2$	53·1	6·9	7·0	52·9	7·0	7·0
H	CH_3O	OH	H	H	1	202	Cl	$C_9H_{14}ClNO_2$	53·1	6·9	7·0	53·3	6·9	6·7
H	OH	OH	H	H	0	228–230	Cl	$C_7H_{10}ClNO_2$	47·8	5·7	8·0	47·2	5·6	8·1
H	OH	OH	H	H	2	156–157	Br	$C_9H_{14}BrNO_2$	44·8	6·3	—	44·5	6·4	—
H	OH	OH	H	H	3	205–207	Br	$C_{10}H_{16}BrNO_2$	45·8	6·2	5·3	46·1	6·1	5·1
H	OH	OH	H	H	4	110–112	Br	$C_{11}H_{18}BrNO_2$	47·8	6·6	5·1	47·8	6·5	5·2
H*	CH_3O	CH_3O	H	CH_3	1	187–188	Cl	$C_{12}H_{20}ClNO_2\frac{1}{2}\cdot H_2O$	56·6	8·3	—	56·8	8·7	—
H†	OH	OH	H	CH_3	1	154–155	—	$C_{10}H_{15}NO_2$	66·3	8·3	—	66·1	8·6	—
H	CH_3O	OH_3	CH_3	H	1	140	Cl	$C_{10}H_{16}ClNO_2$	55·2	7·4	6·4	54·8	7·1	6·4
H	OH	CH_3O	CH_3	H	1	260	Cl	$C_{10}H_{16}ClNO_2$	55·2	7·4	6·4	54·9	7·3	6·4
H	Cl	OH	CH_3	H	1	177	Br	$C_9H_{13}BrClNO$	40·5	4·9	5·3	40·2	4·9	5·4
OH	CH_3	CH_3O	CH_3	H	1	198	Cl	$C_{11}H_{18}ClNO_2$	57·0	7·8	6·0	56·9	7·7	6·1
CH_3O	CH_3	OH	CH_3	H	1	184	Cl	$C_{11}H_{18}ClNO_2$	57·0	7·8	6·0	57·0	7·6	5·9
H	OH	OH	CH_3	H	1	182–184	Br	$C_9H_{14}BrNO_2$	43·6	5·7	5·7	43·6	5·5	5·4
OH	CH_3	OH	CH_3	H	1	230	Cl	$C_{10}H_{16}ClNO_2$	55·2	7·4	6·4	55·4	7·5	6·6

* Methiodide, m.p. 230–231°. (Found: C, 44·1; H, 6·6%. $C_{13}H_{22}INO_2$ requires C, 44·5; H, 6·3%).
† Methiodide, m.p. 200–201°. (Found: C, 40·9; H, 5·7%. $C_{11}H_{18}NO_2$ requires C, 40·9; H, 5·6%).

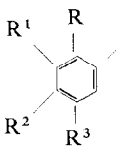
METHODS

Materials. Caeruloplasmin from human plasma was obtained from A.G. Kabi Ltd. (Sweden) as a 5% aqueous solution. The compounds studied as potential substrates were either obtained from commercial sources or, when not commercially available, were synthesized at the Chemical Defence Establishment. In the latter case the structures of all intermediates and end products were verified by infra-red and nuclear magnetic resonance spectroscopy and by melting points where appropriate; in addition for end products not previously described elemental microanalyses were obtained, with results listed in Table 1.

Enzyme studies. The rate of oxygen uptake during the reactions was measured polarographically using a modified Clark electrode,[9] built at CDE, in conjunction with a Radiometer Polarograph Type PO4. The rates of formation of coloured oxidation products from potential substrates (10^{-2}–10^{-5} M) were obtained by measuring the increase in optical density at the λ_{max} using a Perkin-Elmer 137 UV-visible spectrophotometer; the λ_{max} was determined by recording the absorption spectrum of the oxidation product in a preliminary experiment, using the same spectrophotometer. The λ_{max} values are recorded in the appropriate tables. The oxidation of reduced nicotineamide adenine dinucleotide (NADH) to oxidized nicotineamide adenine dinucleotide (NAD^+) by caeruloplasmin in the presence of these compounds (10^{-2}–10^{-5} M) was measured by following the rate of formation of NAD^+ polarographically using a Differential Cathode Ray Polarograph Type A1660 (Southern Analytical Instruments). Some compounds (modifiers) had very low or zero substrate activity but proved capable of modifying the oxidation of noradrenaline and 5-hydroxytryptamine. These effects were studied by measuring the rate of oxidation of noradrenaline or 5-hydroxytryptamine (both 10^{-3} M in 5 ml of pH 5·9 acetate buffer) in the presence of the modifier (10^{-2}–10^{-4} M), the oxidation being initiated by addition of 0·01 ml of 5% aqueous caeruloplasmin to the above solution. The detailed experimental procedures have been described previously.[1]

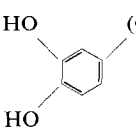
RESULTS

The results are summarized in Tables 2–7. In those cases where a coloured species was formed the K_m and λ_{max} values are given in the Remarks column, the K_m values, which showed a $\pm$ 10% variation on repeated determinations, being based on the rate of increase in optical density at the λ_{max} of the coloured oxidation product. When a compound did not give a coloured species but did consume oxygen and caused the oxidation of NADH to NAD^+ in the presence of caeruloplasmin the K_m values quoted are based on the rates of oxidation of NADH; it must be emphasized, however, that these K_m values do not necessarily possess the same mechanistic significance as those based on the rate of colour formation. The rate of oxidation of compounds XVII–XX and XXXII–XXXIV was too slow to permit accurate measurement of K_m values. Compound IX was oxidized to a coloured material, presumably an aminochrome, which itself inhibited the enzyme. Compound XXII was oxidized by this enzyme, as shown in Table 4, but the coloured species formed had only a weak broad absorption band around 340 nm and an accurate measurement of the K_m value was not possible. However, some indication of its affinity for the enzyme is derived from

TABLE 2. SUBSTITUTED PHENYLETHYLAMINES  AS SUBSTRATES FOR CAERULOPLASMIN†

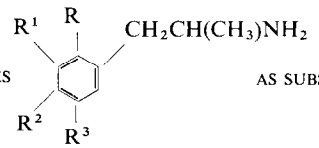
Compound No.	Substituents R	R^1	R^2	R^3	Substrate action O_2 uptake	NADH oxidation	Remarks
I	H	H	H	H	−	−	
II	H	H	OH	H	−	−	
III	H	OH	OH	H	+	+	Coloured pigment formed. λ_{max} = 490 nm, $K_m = 2 \times 10^{-4}$ M
IV	H	CH_3O	CH_3O	CH_3O	−	−	
V	H	CH_3O	CH_3O	H	−	−	
VI	H	OH	H	H	−	−	
VII*	H	OH	CH_3O	H	−	+	$K_m = 3{\cdot}5 \times 10^{-4}$ M
VIII*	H	CH_3O	OH	H	−	+	$K_m = 1{\cdot}2 \times 10^{-3}$ M
IX	OH	H	OH	OH	+	+	Coloured pigment formed. λ_{max} = 500 nm, $K_m = 1 \times 10^{-4}$ M
X	H	OH	OH	OH	+	+	Coloured pigment formed. λ_{max} = 430 nm, $K_m = 1{\cdot}5 \times 10^{-4}$ M

* K_m quoted is based on rate of oxidation of NADH.
† 0·01 ml of a 5% solution of caeruloplasmin in 5 ml of pH 5·9 acetate buffer containing 10^{-2}–10^{-5} M substrate.

TABLE 3. SUBSTITUTED PHENYLALKYLAMINES  AS SUBSTRATES FOR CAERULOPLASMIN*

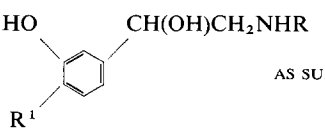
Compound No.	n	Substrate action O_2 uptake	NADH oxidation	Remarks
XI	1	+	+	Coloured pigment formed. λ_{max} = 390 nm, $K_m = 5 \times 10^{-4}$ M
III	2	+	+	Coloured pigment formed. λ_{max} = 490 nm, $K_m = 2 \times 10^{-4}$ M
XII	3	+	+	Coloured pigment formed. λ_{max} = 525 nm, $K_m = 4 \times 10^{-5}$ M
XIII	4	+	+	Coloured pigment formed. λ_{max} = 530 nm, $K_m = 2{\cdot}5 \times 10^{-4}$ M
XIV	5	+	+	Coloured pigment formed. λ_{max} = 400 nm, $K_m = 4 \times 10^{-4}$ M

* 0·01 ml of a 5% aqueous solution of caeruloplasmin in 5 ml of pH 5·9 acetate buffer containing 10^{-2}–10^{-5} M substrate.

TABLE 4. SUBSTITUTED AMPHETAMINES  AS SUBSTRATES FOR CAERULOPLASMIN*

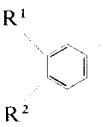
Compound No.	Substituents R	R^1	R^2	R^3	Substrate action O_2 Uptake	NADH oxidation	Remarks
XV	H	H	H	H	−	−	
XVI	H	CH_3O	OH	H	−	−	Inhibits oxidation of noradrenaline and 5-HT. $K_i = 4 \times 10^{-3}$ M.
XVII	H	OH	CH_3O	H	+	+	At concentrations of 3–5 × 10^{-3} M these compounds accelerate oxidation of noradrenaline by 50% but inhibit oxidation of 5-HT by 50%.
XVIII	H	OH	Cl	H	+	+	
XIX	H	OH	CH_3	H	+	+	
XX	CH_3O	H	CH_3	OH	+	+	
XXI	OH	H	CH_3	CH_3O	−	−	Inhibits oxidation of noradrenaline and 5-HT. $K_i = 4 \times 10^{-3}$ M.
XXII	OH	H	CH_3	OH	+	−	Coloured pigment formed. $\lambda_{max} = 340$ nm.
XXIII	H	OH	OH	H	+	+	Coloured pigment formed. $\lambda_{max} = 490$ nm, $K_m = 1{\cdot}2 \times 10^{-4}$ M.
XXIV†	H	OH	OH	H	+	+	
XXV‡	H	OH	OH	H	+	+	

* 0·01 ml of a 5% aqueous solution of caeruloplasmin in 5 ml of pH 5·9 acetate buffer containing 10^{-2}–10^{-5} M substrate.
† D-Enantiomer of compound 23.
‡ L-Enantiomer of compound 23.

TABLE 5. ADRENALINE ANALOGUES  AS SUBSTRATES FOR CAERULOPLASMIN*

Compound No.	Substituents R	R^1	Substrate action O_2 uptake	NADH oxidation	Remarks
XXVI	H	OH	+	+	Coloured pigment formed. $\lambda_{max} = 490$ nm, $K_m = 4{\cdot}5 \times 10^{-4}$ M
XXVII	CH_3	OH	+	+	Coloured pigment formed. $\lambda_{max} = 500$ nm, $K_m = 5 \times 10^{-4}$ M
XXVIII	H	CH_3O	−	−	
XXIX	CH_3	CH_3O	−	−	
XXX	i-C_3H_7	OH	+	+	Coloured pigment formed. $\lambda_{max} = 500$ nm, $K_m = 3 \times 10^{-4}$ M

* 0·01 ml of a 5% aqueous solution of caeruloplasmin in 5 ml of pH 5·9 acetate buffer containing 10^{-2}–10^{-5} M substrate.

TABLE 6. SUBSTITUTED PHENYLALANINES  AS SUBSTRATES FOR CAERULOPLASMIN*

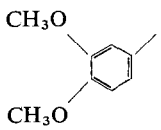
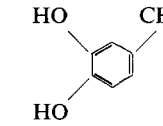
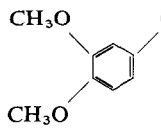
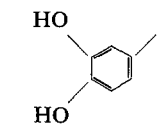
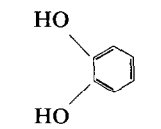
Compound No.	Substituents R	R¹	R²	Substrate action O_2 Uptake	NADH Oxidation	Remarks
XXXI	H	OH	OH	+	+	Coloured pigment formed. λ_{max} = 490 nm. $K_m = 4{\cdot}7 \times 10^{-4}$ M (L) $K_m = 4{\cdot}8 \times 10^{-3}$ M (D)
XXXII	CH_3	OH	H	Very slow	Very slow	
XXXIII	CH_3	OH	OH			
XXXIV	CH_3	CH_3O	H			
XXXV	H	OH	H	−	−	
XXXVI	H	NH_2	OH	+	+	Coloured pigment formed. λ_{max} = 350 nm, $K_m = 2 \times 10^{-4}$ M
XXXVII	H	CH_3O	OH	−	−	
XXXVIII	H	OH	CH_3O	−	−	

* 0·01 ml of a 5% aqueous solution of caeruloplasmin in 5 ml of pH 5·9 acetate buffer containing 10^{-2}–10^{-5} M substrate.

the fact that the oxidation product of XXII is formed even in the presence of noradrenaline, noradrenochrome only being formed when all of the amphetamine has been oxidized; similar comments apply when the substrate used was 5-hydroxytryptamine.

It was reported previously[1,4] that 3-hydroxy-4-methoxyphenyl-ethylamine (compound VII) accelerated the oxidation of noradrenaline, dopamine and 5-hydroxytryptamine whilst the isomeric 3-methoxy-4-hydroxyphenylethylamine (compound VIII) weakly inhibited the oxidation of all three substrates. Similar effects have now been demonstrated with other compounds of the same structural type. Compounds XVII–XX, all of which contain a hydroxy group *m*- to the side chain, were very poor substrates for the enzyme but they all accelerated the oxidation of noradrenaline and inhibited the oxidation of 5-hydroxytryptamine. Attempts to quantify the acceleration of noradrenaline oxidation were unsuccessful since this acceleration became an inhibition at higher concentrations of modifier. The closely related compounds XVI and XXI inhibited the oxidation of both noradrenaline and 5-hydroxytryptamine. The fact that compounds XVII–XX inhibited the oxidation of 5-hydroxytryptamine even though they accelerated the oxidation of noradrenaline contrasts with the previous finding that compound VII, which is structurally closely related to these compounds, accelerated the oxidation of noradrenaline, dopamine and 5-hydroxytryptamine. This material was therefore reinvestigated and it was found that although the rate of oxidation of NADH to NAD^+ was enhanced if this compound was added to an assay solution containing 5-hydroxytryptamine, when allowance was made for the substrate activity of this compound under these conditions (see Table 2) the rate of oxidation of NADH to NAD^+ due to the presence of 5-hydroxytryptamine was actually decreased by compound VII.

TABLE 7. MISCELLANEOUS COMPOUNDS

Compound No.	Structure	Substrate action: O_2 Uptake	Substrate action: NADH oxidation	Remarks
XXXIX		−	−	
XL		+	+	$K_m = 1{\cdot}5 \times 10^{-4}$ M
XLI		−	−	
XLII		+	+	$K_m = 1{\cdot}0 \times 10^{-4}$ M
XLIII		+	+	$K_m \sim 4 \times 10^{-1}$ M
XLIV	Apomorphine	+	+	Coloured pigment formed. Peaks at 420 nm and 630 nm. $K_m = 9 \times 10^{-6}$ M

DISCUSSION

The results listed in Tables 2–7 show quite clearly that one important structural feature for high activity amongst phenylalkylamine-type substrates of caeruloplasmin is the presence, in the aromatic ring, of a 3,4-dioxygenation pattern. Of the two oxygen atoms directly attached to the aromatic ring one must be present as a free hydroxy group whilst the other may be either a free hydroxy group or a methoxy group; a coloured oxidation product is formed, however, only when both oxygen atoms are present as free hydroxy groups. A hydroxy group in position 3 is associated with higher substrate activity than one in position 4 as can be seen by comparing 3-hydroxy-4-methoxyphenylethylamine with 3-methoxy-4-hydroxyphenylethylamine (compounds VII and VIII, Table 2); in fact compound VII compares favourably as a substrate with the corresponding dihydroxy compound (dopamine, compound III, Table 2). However, since the K_m values for compounds III and VII were obtained by two different methods, the interpretative significance of this comparison must await the results of more detailed kinetic studies on these and related compounds. The corresponding pair of amphetamines (compounds XVI and XVII, Table 4) also

illustrate this point, compound XVII, the amphetamine analogue of VII, being a substrate for caeruloplasmin, albeit a poor one, whilst compound XVI, the amphetamine analogue of VIII, showed no substrate activity. It is also clear that a 2,5-dioxygenation pattern is compatible with substrate activity (see compound XXII, Table 4), but synthetic difficulties precluded a more detailed study of this observation at this stage.

The present work has also shown that the basic side chain of catecholamines is necessary for a compound to show high substrate activity. This is most clearly seen by comparing catechol (compound XLIII, Table 7) with, for example, dopamine (compound III, Table 2) or with the compounds in Table 3. Substitution on the side chain nitrogen atom does not appear to have marked effects, as illustrated by a comparison of dopamine (compound III, Table 2) with its *N*,*N*-dimethyl derivative (compound XL, Table 7) and by comparing noradrenaline (compound XXVI, Table 5) with adrenaline (compound XXVII, Table 5) and isoprenaline (compound XXX, Table 5). It is of interest that even quaternization of the side chain has little effect on substrate activity (compare compounds XL and XLII, Table 7), indicating that the nitrogen atom of the side chain interacts in its onium form with the enzyme and not as the uncharged species. This is perhaps not unexpected since these reactions were carried out at a pH (5·9) at which the basic nitrogen of the non-quarternary substrates would effectively be fully protonated.

The effects on substrate activity of varying the distance between the aromatic ring and the basic group of the side chain have been studied, with results shown in Table 3. Although substrate activity was at a maximum when three methylene groups were incorporated into the side chain (3,4-dihydroxyphenylpropylamine, compound XII, Table 3) all these compounds were good substrates for caeruloplasmin. It is therefore clear that whilst the presence of a basic side chain is necessary for high substrate activity the distance separating this basic group from the aromatic ring is not a critical factor in determining substrate activity.

The effects on substrate activity of substitution into the side chain of phenylalkylamines have been investigated using amphetamines (Table 4) and phenylalanines (Table 6). The results in Table 4 indicate that introduction of a methyl group α to the basic group of the side chain has very little effect on substrate activity except in the case of the amphetamine analogues of compounds VII and VIII (Table 2). In these cases introduction of an α methyl group either abolishes substrate activity (compound XVI) or reduces it to such a low level that a K_m value could not be measured (compound XVII). Introduction of a carboxyl group α to the side chain nitrogen atom is associated with a small decrease in substrate activity (see Table 6); additional substitution by a methyl group at this point reduces substrate activity to a very low level (see compound XXXIII, Table 6). It is of interest that 3-aminotyrosine (compound XXXVI), which contains a nuclear amino group in place of the 3-hydroxy group of dopa (compound XXXI), is a better substrate than dopa; it would be of some interest to examine the amino analogues of catecholamines as substrates for this enzyme.

The effect of asymmetry on substrate activity has been studied briefly, with somewhat ambiguous results. In the case of compound XXXI there was a 10-fold difference in K_m for the two enantiomers, the L-enantiomer being the better substrate, but in the case of the one amphetamine, 3,4-dihydroxyamphetamine which was studied in this

way (compounds XXIII–XXV, Table 4) the K_m values for the racemate (compound XXIII) and the two enantiomers (D, compound XXIV; L, compound XXV) were identical. No explanation for this unexpected result can be offered at the present time, although it may be that the charged carboxyl group in the enantiomers of compound XXXI may orientate the side chain in a way which differs from that in which the side chain of the amphetamines interacts with the enzyme. Information on this point may be obtained by studying the esters derived from compound XXXI.

The observation that some of the compounds described in this report can modify the enzymic oxidation of noradrenaline and 5-hydroxytryptamine is of considerable interest. The four substituted amphetamines 3-hydroxy-4-methoxy; 3-hydroxy-4-chloro-, 3-hydroxy-4-methyl-, and 3-methoxy-4-methyl-5-hydroxy amphetamine (compounds XVII–XX, Table 4) accelerated the oxidation of noradrenaline but inhibited the oxidation of 5-hydroxytryptamine; however, at concentrations higher than $3–5 \times 10^{-3}$ M these compounds inhibited the oxidation of both substrates. These differential effects on the oxidation of the two substrates prompted a re-investigation of 3-hydroxy-4-methoxyphenylethylamine (compound 7, Table 2) which it had previously been reported[1,4] accelerated the oxidation of noradrenaline, dopamine and 5-hydroxytryptamine. When due allowance was made for the substrate activity of compound VII (see Table 2) it was found that, like the above compounds, it inhibited the oxidation of 5-hydroxytryptamine. The effects of compounds XVII–XX on the oxidation of 5-hydroxytryptamine are readily observed because these effects are not masked by the substrate activity of these compounds, which is of a very low order; the effects of all five compounds on the oxidation of noradrenaline are also very readily distinguished since none of these compounds give a coloured species under the conditions used for monitoring the rate of oxidation of noradrenaline. The structural requirements related to these differential effects are quite stringent; for example 3-methoxy-4-hydroxyamphetamine and 2-hydroxy-4-methyl-5-methoxyamphetamine (compounds XVI and XXI, Table 4), which are isomeric with compounds XVII and XX respectively, inhibit the oxidation of both noradrenaline and 5-hydroxytryptamine.

The basis for the differential effects of compounds VII and XVII–XX on the oxidation of noradrenaline and 5-hydroxytryptamine requires some consideration. It has previously been suggested[10,11] that caeruloplasmin possesses two catalytically active sites and this proposal finds support in the work of Curzon and Speyer[12] on the effects of inorganic ions on the oxidation of *N,N*-dimethyl-*p*-phenylenediamine by caeruloplasmin and by studies on the binding of transition metal ions to this enzyme.[13] The differential effects of the above compounds on the caeruloplasmin-catalysed oxidation of noradrenaline and 5-hydroxytryptamine also support this concept of two enzymatically active sites and further suggest that, of these two sites, one may be responsible for the oxidation of catecholamines whilst the oxidation of tryptamines may be mediated by the other site. If caeruloplasmin does possess two independent active sites then the effects of those compounds which modify the enzymic oxidation of noradrenaline and 5-hydroxytryptamine may be due to an allosteric modification of enzyme activity. However, an alternative possibility is that whilst caeruloplasmin possesses two distinct substrate binding sites, for phenylalkylamines and tryptamines respectively, there may be only one oxidative site, shared by these two substrate binding sites, which is responsible for the chemical transformation of both types of

substrates. If this is the case then it may be expected that those compounds which modify the oxidation of noradrenaline and 5-hydroxytryptamine do so by interacting with the tryptamine binding site in such a way as to prevent access of 5-hydroxytryptamine (resulting in inhibition) but at the same time either enhancing the interaction of phenylalkylamines with their binding site or enhancing the reactivity of the oxidative site (resulting in an increased rate of oxidation of noradrenaline). It might be anticipated, therefore, that the inhibition of 5-hydroxytryptamine oxidation would be competitive and that the enhanced rate of oxidation of noradrenaline would be reflected by a decrease in K_m if these compounds modify the binding of noradrenaline to the enzyme or by an increase in V_{max} if they modify the reactivity of the oxidative site. Additional detailed kinetic studies necessary to differentiate between these various possibilities are planned. Since several steps are involved in the oxidation of a substrate by caeruloplasmin,[14] and since it is possible that two enzymatically active sites are involved, it seems likely that those compounds described in this paper which differentially affect the oxidation of noradrenaline and 5-hydroxytryptamine may be of use in exploring in detail the mechanism of action of caeruloplasmin at the molecular level.

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