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PHENOL OXIDASE ACTIVITY IN BRAIN TISSUE

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Abstract—An oxidase capable of catalysing the oxidation of aromatic hydroxy and amino compounds is shown to be present in brain tissue. Substrates for this enzyme include *N,N*-dimethyl-*p*-phenylenediamine, dopamine, noradrenaline, 3-(3,4-dihydroxyphenyl)propylamine and 5-hydroxytryptamine. Lysergic acid *N,N*-diethylamide (LSD) accelerated the oxidation of noradrenaline by this enzyme but inhibited the oxidation of 5-hydroxytryptamine. The enzymic oxidation of both noradrenaline and 5-hydroxytryptamine was accelerated by chlorpromazine but was inhibited by imipramine. These results are discussed in terms of a potential biochemical function which this enzyme may have in the maintenance of normal function.

PREVIOUS PAPERS[1,2] have described the effects of certain centrally active drugs on the oxidation of noradrenaline, dopamine and 5-hydroxytryptamine by the copper-containing oxidase caeruloplasmin. Although centrally active glycollates[3] and amphetamines[4] had no effect on the caeruloplasmin-catalysed oxidation of these biogenic amines it was found that lysergic acid *N,N*-diethylamide (LSD) inhibited the enzymic oxidation of 5-hydroxytryptamine but accelerated the oxidation of noradrenaline and dopamine;[1] 3-hydroxy-4-methoxyphenylethylamine and some phenolic amphetamines had similar effects.[1,5] It was also shown that the enzymic oxidation of dopamine, noradrenaline and 5-hydroxytryptamine was accelerated by some tranquillizers, including phenothiazines, but was inhibited by tricyclic antidepressants such as imipramine.[1,2] These observations suggested[1,2] that caeruloplasmin, or an enzyme with similar properties, could represent one of the biochemical mechanisms by which the relative levels of noradrenaline, dopamine and 5-hydroxytryptamine are controlled in those areas of the brain where these amines function as neurotransmitters. Thus 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 interference with this enzyme might result in the appearance of abnormal mental states. It therefore seemed necessary to continue this work along two independent lines. In the first place, a more extensive definition of the substrate specificity of caeruloplasmin, with particular reference to substrates related to catecholamines and tryptamines, seemed desirable. The results of these studies have already been reported.[5,6] In addition it was obviously necessary to determine whether the central nervous system contained an enzyme which was identical with, or very similar to, caeruloplasmin. The present paper gives an account of work leading up to the successful demonstration that an enzyme with the required properties is present in the central nervous system.

METHODS

Materials

Beef and sheep brains were obtained from freshly killed animals and transported in ice to the laboratory. The caudate nucleus and substantia nigra were removed by dissection and used as the starting materials for the extraction procedures outlined below. The substrates and drugs used were obtained either from commercial sources or were synthesized at the Chemical Defence Establishment.[5]

Extraction procedures

The extraction procedures adopted led to the demonstration that two types of catecholamine oxidase activity, operative at pH 9·0 and 5·9 respectively, were present in brain tissue. These two types of activity will be considered separately and, since the enzymes responsible have not as yet been exhaustively purified and characterized, will be referred to by the non-committal names "pH 9·0 enzyme" and "pH 5·9 enzyme".

pH 9·0 enzyme. The wet wt of the caudate nucleus and substantia nigra was determined and the tissue was then homogenized in 5 vol of a solution containing KCl (3 M), K_2HPO_4 (0·15 M) and KH_2PO_4 (0·15 M), pH 9 and the resulting homogenate was centrifuged (35,000 *g*, 1 hr). The residue from this centrifugation was used for the isolation of the pH 5·9 enzyme (see below) whilst the supernatant was treated according to the method of Inchiosa[7] up to the stage at which actinomycin was removed by electrostatic precipitation at pH 5·25. The solution obtained at this stage was assayed at both pH 9·0 and 5·9 for oxidative activity towards catecholamines; oxidase activity was observed only at pH 9·0.

pH 5·9 enzyme. The residue from the first centrifugation of the tissue homogenate (see above) was resuspended in distilled water and an acetone powder prepared by carefully pouring this suspension into 10 volumes of acetone at −20°. The precipitate was collected by filtration, resuspended with stirring in *n*-butanol at −5° and again collected by filtration, the residue being washed on the filter with acetone at −20° and finally dried *in vacuo*. This acetone powder was rehomogenized in 0·9% NaCl and the suspension centrifuged (35,000 *g*, 1 hr); the centrifugate was resuspended in acetate buffer (0·05 M, pH 5·9) and centrifuged (35,000 *g*, 1 hr) to give a supernatant which was then dialysed against distilled water. An aliquot of the dialysate was assayed at both pH 9·0 and pH 5·9 for oxidase activity towards catecholamines; oxidase activity was observed only at pH 5·9. The remainder of this dialysed supernatant fluid was lyophilized and the resulting solid (approx. 5 mg from four beef brains) was stored at 0°. At this temperature the oxidase activity was only slowly lost over a period of several weeks; warming a portion of this material, redissolved in acetate buffer at pH 5·9, to 100° for 30 min resulted in complete loss of activity. A flow diagram of these extraction procedures is shown below.

Enzyme studies

Substrate studies. The oxidase activity of the two preparations towards catecholamines and *N,N*-dimethyl-*p*-phenylenediamine was measured at pH 9·0 and 5·9 by taking repetitive spectra, over the range 270–670 nm, at 5 min intervals using a Perkin–Elmer 402 spectrophotometer the cells of which were maintained at 37°. The oxidation of 5-hydroxytryptamine was measured by monitoring the rate of conversion of reduced

nicotineamide adenine dinucleotide (NADH) to oxidized nicotineamide adenine dinucleotide (NAD^+) by the enzyme in the presence of 5-hydroxytryptamine,[8] using the change in absorbance at 340 nm, recorded on the Perkin–Elmer 402 spectrophotometer at 37°, as a measure of the change in concentration of NADH. Oxygen uptake during these reactions was monitored using a Clark electrode[9] inserted into the spectrophotometric cell.

Effect of drugs on oxidase activity. Since the pH 9·0 enzyme did not catalyse the oxidation of 5-hydroxytryptamine only catecholamine-type substrates could be used in this aspect of the investigation. The substrates used were adrenaline and 3-(3,4-dihydroxyphenyl) propylamine; the effects of added drugs on the enzymic oxidation of these two substrates were obtained from repetitive spectra recorded over the spectral range 270–670 nm at 5 min intervals, using the Perkin–Elmer 402 spectrophotometer, in the presence and absence of the drug.

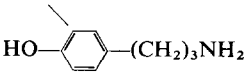
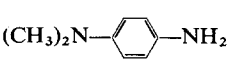
The effects of various drugs on the oxidase activity of the pH 5·9 enzyme, using noradrenaline as the substrate, were obtained by measuring the differences in the rate of change in absorbance at 490 nm in the presence and absence of the drugs, using the Perkin–Elmer 402 spectrophotometer. When 5-hydroxytryptamine was used as the substrate the effect of these drugs on the rate of change in absorption at 340 nm in the presence of NADH[8] was used to monitor the modified enzymic oxidation.

The detailed experimental procedures were essentially those described previously.[1]

RESULTS

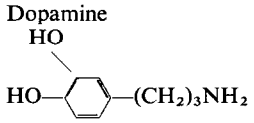
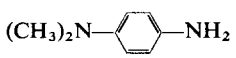
pH 9·0 enzyme. The substrate specificity of this enzyme was determined at pH 9·0, with the results shown in Table 1. Since the enzyme preparation was at this stage relatively crude it was felt that K_m and V_{max} values would have no real mechanistic significance; consequently the results are expressed relative to adrenaline = 100 arbitrary units. In contrast to caeruloplasmin and the pH 5·9 enzyme 5-hydroxytryptamine and *N,N*-dimethyl-*p*-phenylenediamine were not substrates for this enzyme nor did the enzyme convert NADH into NAD^+ in the presence of these substrates. This preparation had no measurable oxidase activity at pH 5·9.

TABLE 1. SUBSTRATE SPECIFICITY OF pH 9·0 ENZYME

Substrate	Oxidase activity, adrenaline = 100	
	At 490 nm	At 390 nm
Adrenaline	100	100
Noradrenaline	20	20
Dopamine	45	30
	330	20
	0	0
5-Hydroxytryptamine	0	0

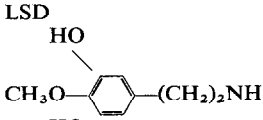
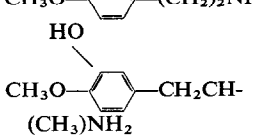
pH 5·9 enzyme. The substrate specificity of this preparation was determined at pH 5·9, with the results shown in Table 2. For the reasons given above K_m and V_{max} values were not determined at this stage and Table 2 therefore merely indicates which of the compounds tested acted as substrates. Although molecular oxygen was utilized during the enzyme assays no peroxide was formed. In contrast to caeruloplasmin the oxidation of ferrous ion to ferric ion was not catalysed by this preparation.

TABLE 2. SUBSTRATE SPECIFICITY OF pH 5·9 ENZYME

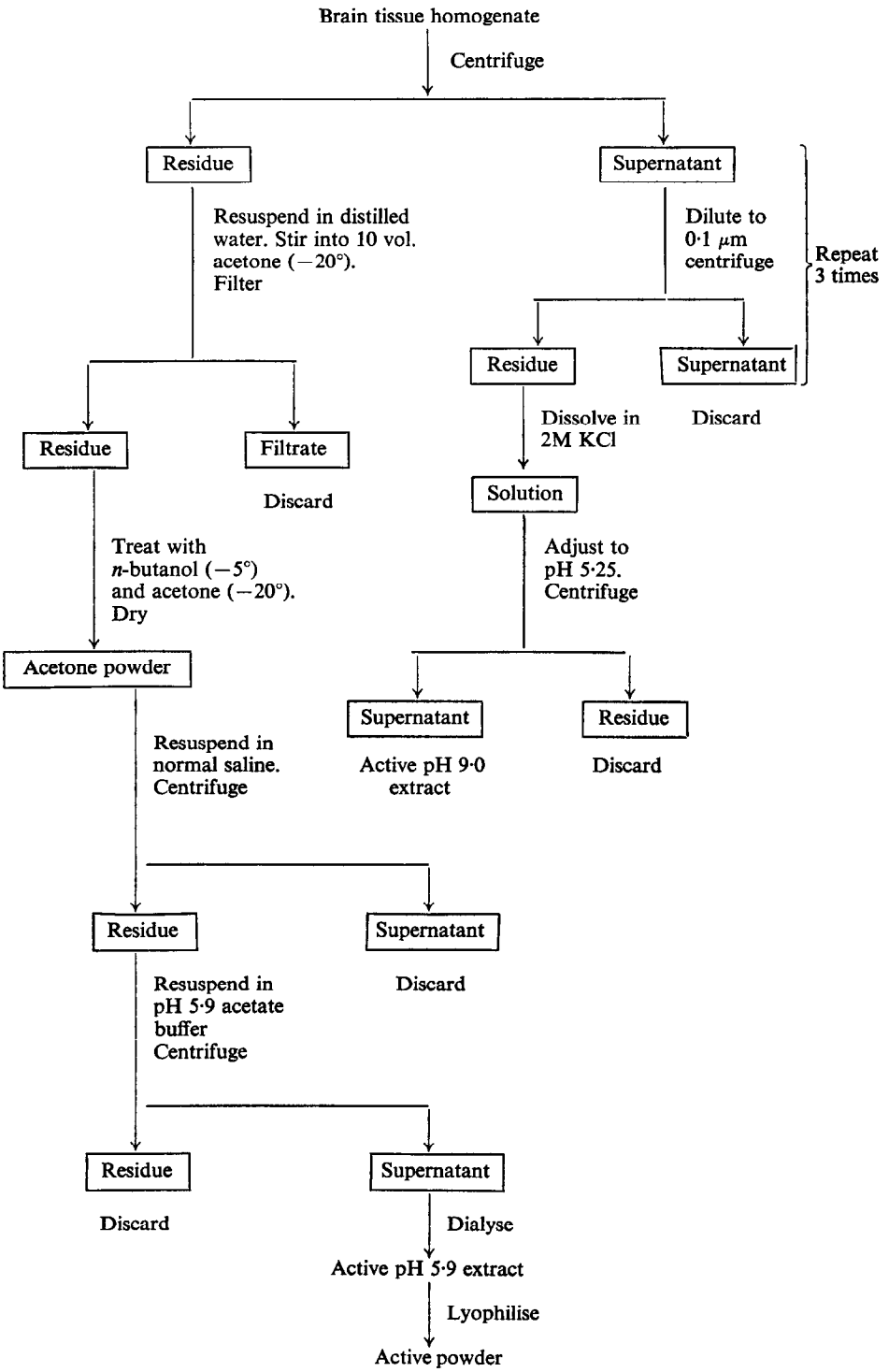
Substrate	Oxidase activity	
	Aminochrome formation	NADH oxidation
Adrenaline	+	+
Noradrenaline	+	+
Dopamine	+	+
	+	+
	+	−
5-Hydroxytryptamine	−	+

The effects of centrally active drugs on the oxidation of noradrenaline and 5-hydroxytryptamine by this preparation were studied at pH 5.9, with the results shown in Table 3. The acceleration of noradrenaline oxidation which was observed with 3-hydroxy-4-methoxyphenylethylamine and the corresponding isopropylamine derivative changed to inhibition with increased drug concentration or decreased substrate concentration.

TABLE 3. EFFECT OF ADDITIVES ON THE OXIDATION OF NORADRENALINE AND 5-HYDROXYTRYPTAMINE BY THE pH 5·9 ENZYME

Additive	Effect on oxidation of substrate	
	Noradrenaline	5-Hydroxy-tryptamine
LSD	Acceleration	Inhibition
	Acceleration	Inhibition
	Acceleration	Inhibition
Chlorpromazine	Acceleration	Acceleration
Imipramine	Inhibition	Inhibition

PREPARATION OF pH 9·0 AND pH 5·9 OXIDASES FROM MAMMALIAN BRAIN



DISCUSSION

The studies reported in this paper resulted directly from the suggestion[1,2] that a phenol oxidase, which could be of importance in the regulation of mental function, is present in the central nervous system. The properties which such an oxidase should possess may be defined on the basis of previous studies[1,2,5] with some degree of precision. Thus it should be capable of catalysing the oxidation of dopamine, noradrenaline and 5-hydroxytryptamine and the oxidation of the catecholamine substrates should be affected by centrally active drugs in a way which differs from their effects on the oxidation of 5-hydroxytryptamine.

Previous workers have demonstrated the presence of catecholamine oxidase activity in central nervous tissue[10–13] but the modification of this activity by other drugs has not been reported. Using an extraction procedure similar to that described by Inchiosa[7,13,14] it was possible to isolate an enzyme preparation which possessed oxidase activity, at pH 9·0, towards catecholamines but which did not catalyse the oxidation of 5-hydroxytryptamine or *N*,*N*-dimethyl-*p*-phenylenediamine (see Table 1). This enzyme preparation did not catalyse the oxidation of NADH to NAD^+ in the presence of noradrenaline, as does caeruloplasmin,[8] and its oxidase activity towards adrenaline and 3-(3,4-dihydroxyphenyl) propylamine was not modified by LSD or by 3-hydroxy-4-methoxyphenylethylamine. It is therefore clear that this enzyme, which is probably identical with that isolated from brain tissue by Inchiosa,[14] does not possess the properties necessary for it to fulfil the postulated[1,2] regulatory role in those areas of the brain where dopamine, noradrenaline and 5-hydroxytryptamine act as neurotransmitters

Work on the residue obtained during the first stage of the isolation of this enzyme (see flow diagram) resulted in the separation of a fraction possessing a second type of catecholamine oxidase activity This fraction possessed oxidase activity at pH 5·9, as does caeruloplasmin; in addition, like caeruloplasmin, it catalysed the oxidation of catecholamines, 5-hydroxytryptamine and *N*,*N*-dimethyl-*p*-phenylenediamine (see Table 2). A further point of resemblance to caeruloplasmin was the fact that it catalysed the oxidation of NADH to NAD^+ but only in the presence of catecholamine substrates or 5-hydroxytryptamine.[8] Unlike caeruloplasmin there was no evidence that this enzyme catalysed the oxidation of ferrous ion to the ferric state. The effects of various centrally active drugs on the oxidation of noradrenaline and 5-hydroxytryptamine by this enzyme preparation (see Table 3) were identical with their effects on the caeruloplasmin-catalysed oxidation of these two substrates. Thus, LSD, 3-hydroxy-4-methoxyphenylethylamine and the corresponding isopropylamine derivative inhibited the oxidation of 5-hydroxytryptamine and accelerated the oxidation of noradrenaline; the oxidation of both substrates was accelerated by the tranquillizer chlorpromazine and inhibited by the antidepressant drug imipramine. It would thus seem that the oxidative enzyme present in this fraction closely resembles but is probably not identical with caeruloplasmin. Certainly it possesses those properties which would enable it to act as one of the regulatory mechanisms which control the relative levels of dopamine, noradrenaline and 5-hydroxytryptamine in those areas of the brain where these amines function as neurotransmitters. It is therefore possible that this pH 5·9 enzyme may play a significant role in the maintenance of normal mental function and that modification of this enzyme activity may lead to abnormal mental conditions.

It has been noted (Table 3) that 3-hydroxy-4-methoxyphenylethylamine accelerates the enzymic oxidation of noradrenaline but inhibits the oxidation of 5-hydroxytryptamine; *in vivo* it might therefore be anticipated that this compound would alter the balance between dopamine, noradrenaline and 5-hydroxytryptamine It is known that *in vitro* catecholamine *O*-methyltransferase will catalyse the methylation of the 4-hydroxy group of catechols as well as the 3-hydroxy group,[15,16] although the latter reaction normally preponderates. It has also been shown[17] that one of the expected metabolites of 3-hydroxy-4-methoxyphenylethylamine, the corresponding phenylacetic acid (homoisovanillic acid), occurs in cerebrospinal fluid. Thus there is circumstantial evidence that 3-hydroxy-4-methoxyphenylethylamine is produced *in vivo* as a normal, although minor, metabolite of dopamine.[17] It would therefore appear legitimate to ask whether this compound might be the endogenous psychotoxin responsible, at least in part, for the onset of mental illness, particularly schizophrenia. Since there is evidence that 3-hydroxy-4-methoxyphenylethylamine is a normal minor metabolite of dopamine the significant demonstration would not only be that it occurs in brain tissue but that its level, relative to 3-methoxy-4-hydroxyphenylethylamine, was elevated in schizophrenic individuals. Support for this suggestion could perhaps most readily be obtained by measuring the ratio of homoisovanillic to homovanillic acid in the tissues or body fluids of patients suffering from mental illness.

Finally it is perhaps worth emphasizing that these suggestions regarding the possible role of 3-hydroxy-4-methoxyphenylethylamine in mental illness do not necessarily solve the problem of the biochemical basis of schizophrenia since possible explanations for the increased levels of this amine must be considered It has previously been suggested[18] that aberrant methylation is linked with schizophrenia and since 3-hydroxy-4-methoxyphenylethylamine is a methylated derivative of dopamine it is reasonable to suppose that an increase in the ratio of 4-*O*-methylation relative to 3-*O*-methylation, leading to elevated levels of this compound, may result from a modification or malfunction of the enzyme—catecholamine *O*-methyltransferase—which is responsible for the methylation of catecholamines. Support for this suggestion comes from the demonstration,[16] using a series of substituted catechols as substrates, that the ratio of 4-*O*-methylation to 3-*O*-methylation is variable and is markedly dependent on the nature of the substrate; whether this ratio for a particular substrate can also be varied by changes in experimental conditions or by the use of additives was not, however, clearly established. It is possible that this ratio may be modified, in favour of an increased formation of the 4-*O*-methyl metabolite, by other drugs or by a change in the enzyme itself.[19] It is therefore suggested that modification or malfunction of catecholamine *O*-methyltransferase may lead to elevated levels of 3-hydroxy-4-methoxyphenylethylamine which then interacts with the pH 5·9 enzyme described in the present paper to produce an imbalance in the relative levels of dopamine, noradrenaline and 5-hydroxytryptamine, this imbalance being the immediate cause of the onset of mental disturbance.

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