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INTERACTION OF SOME CENTRALLY ACTIVE DRUGS WITH CAERULOPLASMIN

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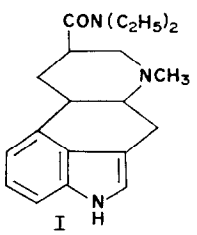
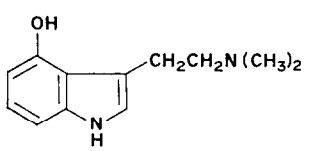
Abstract—Centrally active drugs such as D-lysergic acid diethylamide (LSD), and to a lesser extent ibogaine and 2-bromo-LSD, are shown to inhibit the caeruloplasmin-catalysed oxidation of 5-hydroxytryptamine but accelerate the oxidation of noradrenaline and dopamine. Harmine and harmol inhibit the enzymic oxidation of all three substrates. Centrally active anticholinergics and substituted phenylethylamines do not affect the enzymic oxidation of these substrates. Some drugs used in the treatment of mental illness affect the caeruloplasmin-catalysed oxidation of noradrenaline, dopamine and 5-hydroxytryptamine. Tranquillizers of the phenothiazine class, for example, accelerate the oxidation of all three substrates whilst antidepressant drugs (other than monoamine oxidase inhibitors) inhibit the oxidation of all three substrates. The results obtained show that caeruloplasmin cannot be generally used as a model for those receptors with which some centrally active drugs must interact to produce their characteristic effects; they do, however, suggest that caeruloplasmin, or an enzyme with similar properties, may be of importance in controlling the relative concentrations of noradrenaline, dopamine and 5-hydroxytryptamine in those areas of the brain where these compounds act as neuro-transmitters.

MANY compounds are now known which at low doses will affect mood, perception and behaviour;[1,2] compounds such as D-lysergic acid diethylamide (LSD, I), psilocin (II), mescaline (III), 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM, IV) and some anticholinergics[3] may be cited as examples.

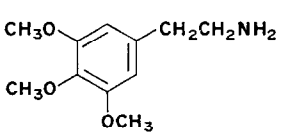
These compounds are of importance to the biologist not only because of their effects on mood, perception and behaviour but also because they offer the possibility of relating such effects to more fundamental molecular events; in other words these compounds can be used as specific chemical probes for studying the central nervous



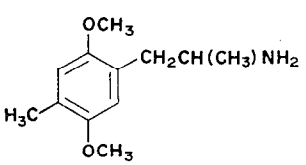
I



II

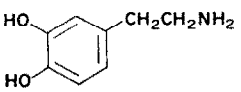


III

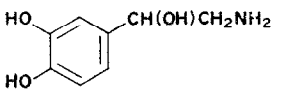


IV

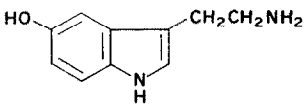
system at the molecular level. Before these compounds can be used in this way, however, it is necessary that their mode of action must be understood in some detail; this essential requirement is not fulfilled by any of the above compounds (I–IV) although the glycollates described by Abood,[3] which have high peripheral anticholinergic activity, probably exert their central effects by interfering with central cholinergic pathways.[4] In view of the chemical similarity between dopamine (V), noradrenaline (VI) and 5-hydroxytryptamine (VII) on the one hand and LSD, psilocin, mescaline and DOM on the other a mode of action for these compounds involving interference with central dopaminergic, noradrenergic or serotoninergic systems seems likely.[5,6]



V



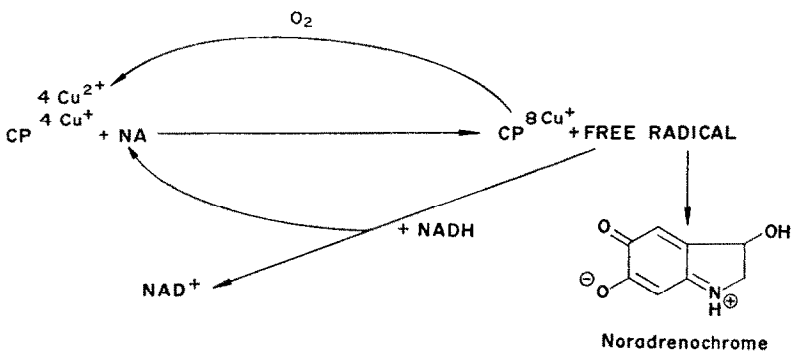
VI



VIII

Certainly in the case of LSD its known peripheral antagonism of 5-hydroxytryptamine[7] has been extensively quoted as supporting the suggestion that its central effects are are due to some kind of interference with an as yet unspecified central serotoninergic system. However, it must be acknowledged that attempts to account fully for the central effects of LSD, mescaline and similar compounds in terms of their interference with the synthesis, uptake, release, antagonisms or destruction of natural transmitters such as noradrenaline, dopamine and 5-hydroxytryptamine have not so far proved wholly satisfactory.

In a search for a possible biochemical investigation into the mode of action of centrally active drugs such as I–IV it was noted that administration of LSD to animals resulted in an elevation of brain 5-hydroxytryptamine levels and a concomitant depression of brain catecholamine levels.[8,9] It therefore seemed to be of interest to study the effects of LSD on an enzyme which utilized both noradrenaline and 5-hydroxytryptamine as substrates since it was considered that such an enzyme might be a useful model for those central receptors with which LSD must interact in order to produce its characteristic central effects. The enzyme which was eventually selected for his study was the copper-containing oxidase caeruloplasmin, which utilizes both noradrenaline and 5-hydroxytryptamine as substrates in the cycle of reactions illustrated by Scheme 1.



SCHEME 1. —CP^{4Cu+}—Oxidized caeruloplasmin, CP^{8Cu^+}—reduced caeruloplasmin, NA—Noradrenaline.

A similar scheme may be drawn in the case of 5-hydroxytryptamine.

In this paper are reported the results of a series of investigations into the effects of different types of centrally active drugs on the caeruloplasmin-catalysed oxidation of noradrenaline, dopamine and 5-hydroxytryptamine.

METHODS

Materials

Caeruloplasmin from human plasma was obtained from A. G. Kabi Ltd. (Sweden) as a 5% aqueous solution. The drugs used were either obtained from commercial sources or were synthesized at the Chemical Defence Establishment following published procedures.

Enzyme studies

(a) *General*

The rate of oxygen uptake during the reactions was measured polarographically using a modified Clark electrode[10] built at CDE. The rate of formation of aminochromes from noradrenaline and dopamine was followed by measuring, spectrophotometrically using a Perkin–Elmer 137 spectrophotometer, the change in absorption at 490 nm. The oxidation of 5-hydroxytryptamine was measured by adding reduced nicotineamide adenine dinucleotide (NADH) to the reaction solution and measuring the rate of change in absorption at 340 nm, due to disappearance of NADH, using the Perkin–Elmer 137 Spectrophotometer. The rate of formation of oxidized nicotineamide adenine dinucleotide (NAD^+) from NADH in the presence of either noradrenaline, dopamine, or 5-hydroxytryptamine was measured polarographically on a Southern Analytical Differential Cathode Ray Polarograph Type A1660.

In the studies on LSD it was necessary to prepare fresh solutions daily as the effects varied with time, possibly due to the slow conversion of LSD into its lumi form.[11]

(b) *Procedures*

Effects of compounds on the oxidation of noradrenaline and dopamine by caeruloplasmin. To a 2×10^{-3} M solution (5 ml) of L-noradrenaline or dopamine in 0·05 M acetate buffer at pH 5·9 was added 0·01 ml of a 5% aqueous caeruloplasmin solution and the mixed solution was placed in a 10-mm path length spectrophotometer cell in the sample compartment of a Perkin–Elmer 137 Spectrophotometer. A similar cell containing only pH 5·9 acetate buffer was placed in the reference compartment. The cell compartments were maintained at 25° and the rate of increase in optical density at 490 nm was monitored using the read-out facility of the spectrophotometer attached to a Beckmann flat-bed recorder. This experiment was repeated using known concentrations of the test compound in 5 ml of 2×10^{-3} M noradrenaline or dopamine in pH 5·9 acetate buffer.

Effects of compounds on the oxidation of reduced nicotineamide, adenine dinucleotide by caeruloplasmin in the presence of substrate. (i) *Spectrophotometrically.* The procedure was essentially as described above using a reaction volume of 5 ml containing 10^{-3} M 5-hydroxytryptamine (or noradrenaline or dopamine) and 2×10^{-4} M reduced nicotineamide dinucleotide in pH 5·9 acetate buffer to which was added 0·01 ml of a 5% aqueous caeruloplasmin solution. The rate of decrease in optical density at 340 nm was monitored as described above.

(ii) *Polarographically.* A solution (5 ml) of 10^{-3} M 5-hydroxytryptamine (or nora-

drenaline or dopamine) containing reduced nicotineamide adenine dinucleotide (5×10^{-4} M) in pH 5·9 acetate buffer was placed in a polarographic cell, maintained at 25°, with a quiet mercury pool anode and a dropping mercury cathode. A 5% aqueous solution (0·01 ml) of caeruloplasmin was then added and the solution mixed by passing a stream of air through it for 5 sec. The cell was then connected to the Cell 1 input of the A1660 Cathode Ray Polarograph and the rate of increase in peak height at −0·9 V, due to the formation oxidised nicotineamide adenine dinucleotide (NAD^+) was recorded; this was expressed as μM NAD^+ produced per minute by reference to a previously constructed calibration curve. This procedure was then repeated with the addition of known concentrations of each of the compounds under study.

Effects of compounds on the rate of oxygen removal. Noradrenaline, dopamine, or 5-hydroxytryptamine (5 ml, 2×10^{-3} M) in pH 5·9 acetate buffer was placed in a thermostatted cell maintained at 25° and a Clark-type oxygen electrode was inserted. The solution was stirred magnetically whilst a potential of +0·6 V was applied to the electrode from a Radiometer PO4 Polarograph. The resulting current was recorded as a function of time using the recorder on the polarograph. After 5 min caeruloplasmin (0·025 ml of a 5% aqueous solution) was added and the current was recorded for a further 20 min. This procedure was repeated with known concentrations of each of the test compounds added to the reaction solution.

Substrate action of test compounds. This was investigated by substituting known concentrations of the test compounds for the standard substrates in the above procedures.

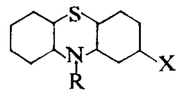
RESULTS

The results obtained are summarized in Tables 1, 2 and 3. LSD, ibogaine and 2-bromo-LSD (BOL) catalysed the enzymic oxidation of dopamine and noradrenaline

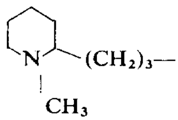
TABLE 1. EFFECTS OF SOME CENTRALLY ACTING INDOLES ON THE OXIDATION OF NORADRENALINE, DOPAMINE AND 5-HYDROXYTRYPTAMINE BY CAERULOPLASMIN

Compound	Action on oxidation of: Noradrenaline and dopamine	Action on oxidation of: 5-Hydroxytryptamine	Comments
LSD	Catalysis. 400% at a concentration 1/10 of substrate	Inhibition. 50% at a concentration 1/10 of substrate	Hallucinogen
Ibogaine	Catalysis. 200% at a concentration equal to substrate	Inhibition. 50% at a concentration equal to substrate	Hallucinogen
2-Bromo-LSD	Catalysis*. 200% at a concentration ½ that of substrate	Inhibition. 50% at a concentration ½ that of substrate	Hallucinogen only at high doses
Harmine	Inhibition. 50% at 10^{-3} M	Inhibition. 50% at 10^{-3} M	Hallucinogenic activity not definitely established
Harmol	Inhibition. 50% at 10^{-4} M	Inhibition. 50% at 10^{-4} M	

* Data for noradrenaline only.

TABLE 2. EFFECTS OF SOME PHENOTHIAZINES 

ON THE OXIDATION OF NORADRENALINE, DOPAMINE AND 5-HYDROXYTRYPTAMINE BY CAERULOPLASMIN

Compound		Action on oxidation of		Comments
R	X	Noradrenaline and dopamine	5-Hydroxy-tryptamine	
$(CH_3)_2N(CH_2)_3$—	Cl	Catalysis. 200% at 10^{-3} M	Catalysis. 200% at 10^{-3} M	Tranquillizer (Chlorpromazine)
 $CH_3N\ \ N(CH_2)_3$—	CF_3	Catalysis. 200% at 2×10^{-4} M	Catalysis. 200% at 2×10^{-4} M	Tranquillizer (Trifluperazine)
$(CH_3)_2N(CH_3)_3$—	CF_3	Catalysis*. 200% at 10^{-3} M	Catalysis. 200% at 10^{-3} M	Tranquillizer (Triflupromazine)
 N $(CH_2)_3$— CH$_3$	SCH_3	Catalysis. 200% at 2×10^{-3} M	Catalysis. 200% at 2×10^{-3} M	Tranquillizer (Thioridazine)
$(CH_3)_2NCHCH_2$— (CH$_3$)	H	No effect	No effect	Anti-histaminic Anti-emetic (Promethazine)
$(C_2H_5)_2N(CH_2)_2$—	H	No effect	No effect	Anti-Parkinsonism (Diethazine)

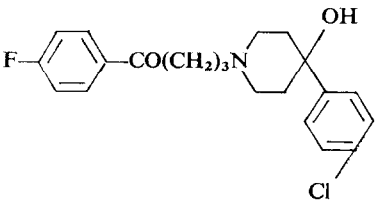
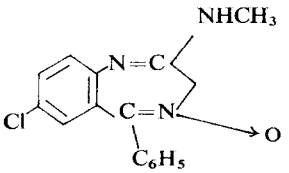
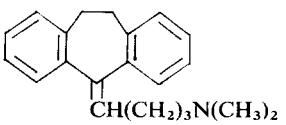
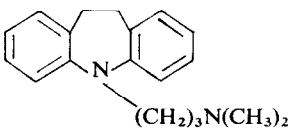
* Data for noradrenaline only.

but inhibited the oxidation of 5-hydroxytryptamine; ibogaine and LSD were also weak substrates for caeruloplasmin. Harmine and harmol inhibited the oxidation of all three substrates.

The catecholaines mescaline, 3,4-dimethoxyphenylethylamine and 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM, IV) had no effects on caeruloplasmin either alone or in the presence of any of the standard substrates. 3-Hydroxy-4-methoxyphenylethylene was a substrate for caeruloplasmin ($K_m = 3{\cdot}5 \times 10^{-4}$ M) and also catalysed the oxidation of noradrenaline, dopamine, and 5-hydroxytryptamine (150% at 2×10^{-3} M for all three substrates); the isomeric 4-hydroxy-3-methoxyphenylethylamine was a poor substrate ($K_m = 1{\cdot}2 \times 10^{-3}$ M) for the enzyme and had no effect on the enzymic oxidation of the standard substrates.

Centrally active anticholinergics of the type described by Abood[3] had no effect on caeruloplasmin either alone or in the presence of noradrenaline, dopamine or 5-hydroxytryptamine.

TABLE 3. EFFECTS OF SOME ANTIDEPRESSANTS AND TRANQUILLIZERS ON THE CAERULOPLASMIN-CATALYZED OXIDATION OF NORADRENALINE, DOPAMINE AND 5-HYDROXYTRYPTAMINE

Compound	Effects on oxidation of		Comments
	Noradrenaline and dopamine	5-Hydroxy-tryptamine	
	Catalysis. 200% at 2×10^{-3} M	Catalysis. 200% at 2×10^{-3} M	Tranquillizer (Haloperidol)
	No effect	No effect	Minor Tranquillizer (Chlordiaz-epoxide)
	Inhibition. 50% at 10^{-2} M	Inhibition. 50% at 10^{-2} M	Antidepressant (Amitryptyline)
	Inhibition. 50% at 10^{-2} M	Inhibition. 50 %at 10^{-2} M	Antidepressant (Imipramine)
	No effect	No effect	Antidepressant (Iproniazid)

Tranquillizers of the phenothiazine and aminobutyrophenone classes accelerated the enzymic oxidation of noradrenaline, dopamine and 5-hydroxytryptamine (Tables 2 and 3) whilst antidepressant drugs, with the exception of iproniazid (a known inhibitor of monoamine oxidase) inhibited the oxidation of all three substrates.

Librium, the phenothiazines Promethazine and Diethazine (which do not possess clinically useful tranquillizing activity) and iproniazid had no effect on the enzymic oxidation of the three standard substrates.

DISCUSSION

The effects of LSD on the caeruloplasmin-catalysed oxidation of noradrenaline, dopamine and 5-hydroxytryptamine have proved to be of considerable interest. The observed inhibition of the enzymic oxidation of 5-hydroxytryptamine by LSD is perhaps not altogether surprising in view of the chemical similarity of the two molecules (I and VII) respectively but the fact that this compound also accelerates the enzymic oxidation of noradrenaline and dopamine was unexpected and raises some interesting points regarding the mechanism of action of caeruloplasmin. Perhaps the most likely explanation for these effects is that the enzyme has two distinct binding sites, for catecholamines and 5-hydroxytryptamine respectively, but that these have the oxidative site in common. LSD would then be considered to interact with the 5-hydroxytryptamine-binding site in a manner which inhibits binding of this particular substrate to the enzyme but which enhances the interaction of catecholamines with their specific binding site. This suggestion that caeruloplasmin has two distinctive active sites is supported by the work of Curzon and Spayer[12] who, using unsymmetrical *N,N*-dimethyl-*p*-phenylenediamine as substrate and inorganic ions as inhibitors concluded that there were two distinct enzymic sites at which these low molecular weight inhibitors could interact. An alternative possibility, that the effects of LSD are wholly allosteric in nature, cannot be ruled out but there is at present no evidence that such effects are involved. Ibogaine and 2-bromo-LSD showed the same effects as LSD but only at higher concentrations whilst harmine and harmol inhibited the oxidation of all three substrates. Although these four compounds have been claimed to possess central activity[13–16] this is only manifest at doses much higher than those required for LSD.[13]

The results obtained with the other centrally active compounds studied were rather more ambiguous. The glycollates described by Abood[3] had no effect on the enzymic oxidation of noradrenaline, dopamine or 5-hydroxytryptamine. This observation is in accord with the suggestion that they exert their central effects by interfering with central cholinergic pathways.[4]

The substituted phenylalkylamines mescaline, 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) and 3,4-dimethoxyphenylethylamine had no effects on caeruloplasmin—they were not substrates nor did they modify the oxidation of known substrates. In other studies on the substrate specificity of caeruloplasmin it was noted that 3-hydroxy-4-methoxyphenylethylamine, in addition to being a substrate for this enzyme, accelerated the oxidation of noradrenaline, dopamine and 5-hydroxytryptamine; the isomeric 4-hydroxy-3-methoxyphenylethylamine was inactive in this respect. Since demethylated metabolites of mescaline have been isolated from rats given this drug[17] it is possible that demethylated metabolites of centrally active phenylalkylamines may be the chemical species which actually elicit the central effects. In such a situation there should be a correlation between the central effects of the compound administered and the interaction of its demethylated metabolite with caeruloplasmin, but there would be no correlation between the central effects of the unmetabolized drugs and their effects on the enzyme. In this context it is significant that a recent report[18] demonstrated the conversion of L-3-*O*-methyl dopa into dopamine

by brain slices indicating the presence, in brain tissue, of an enzyme capable of demethylating drugs of the catecholamine type structure.

Centrally active indoles (*N*-methyl- and *N,N*-dimethyltryptamine) described by Brimblecombe *et al.*[19] had no effect on caeruloplasmin either alone or in the presence of the three substrates. It is possible that with these compounds it is a hydroxylated metabolite which is the active species, as suggested by the above authors[19] and also by Szara.[20] These metabolites would, however, be formed by hydroxylation of the aromatic ring whereas the postulated hydroxy metabolites of compounds such as mescaline and DOM would be formed by demethylation of one of the aromatic methoxyl groups which are so characteristic a feature of the centrally active phenylalkylamines.

These studies have shown that LSD is unique amongst centrally active compounds both as regards the type of effects it has on caeruloplasmin and the low concentrations at which such effects can be demonstrated, leading to the suggestion that this enzyme, or one with similar properties, may be directly implicated in the mode of action of LSD. It is known that the K_m values for the oxidation of noradrenaline and 5-hydroxytryptamine are virtually identical,[21] suggesting the caeruloplasmin or a similar enzyme could exercise a very sensitive control over the relative concentrations of these two amines (and probably also dopamine), in those parts of the brain where they act as neurotransmitters. If, as seems likely, the maintenance of a balance between noradrenaline, dopamine and 5-hydroxytryptamine is essential to normal mental function[6] then LSD could produce its central effects by disturbing the central balance between these biogenic amines as a result of its interaction with caeruloplasmin or a similar enzyme; moreover since LSD affects the oxidation of the catecholamines and 5-hydroxytryptamine in opposite directions it should be more potent than a compound which affects the oxidation of only one of these substrates. This suggestion receives additional support from the observations already referred to[8,9] that administration of LSD to experimental animals is accompanied by a rise in brain 5-hydroxytryptamine levels and a fall in brain catecholamine levels.

Implicit in the above suggestion is the assumption that caeruloplasmin or a similar enzyme is directly involved in the maintenance of normal mental function. Since the investigations so far described have dealt with compounds known to modify normal mental states, it was considered desirable to extend this work to compounds known to be capable of exerting a therapeutically useful effect on abnormal mental states. These results are shown in Tables 2 and 3. Tranquillizers of the phenothiazine class accelerated the enzymic oxidation of noradrenaline, dopamine and 5-hydroxytryptamine, whereas phenothiazines which lack useful tranquillizing properties (Promethazine and Diethazine) had no effect. It is interesting that Haloperidol, which is a tranquillizer of the butyrophenone class, also accelerated the oxidation of these substrates indicating that this effect is a reflection of tranquillizing properties and not of the phenothiazine structure *per se*. Chlordiazepoxide, a very weak tranquillizer used mainly for anxiety states rather than psychotic conditions, had no effect on the enzymic oxidation of these substrates.

The anti-depressants Amitryptyline and Imipramine, and their desmethyl analogues, inhibited the enzymic oxidation of both substrates but only at relatively high concentrations. Again it is significant that Iproniazid, which owes its anti-depressant activity to its known inhibition of monoamine oxidase, had no effect on caeruloplasmin.

The results described in this paper show that caeruloplasmin cannot be used at this stage as a model for those central receptors with which drugs such as LSD, mescaline and DOM must interact in order to produce their central effects. These results do, however, suggest that caeruloplasmin may be directly involved in both the mode of action of centrally active drugs and in the aetiology of some forms of mental illness. Whether these forms of mental illness are due to the effects of endogenously produced compounds which are acting on caeruloplasmin or a similar enzyme must remain an open question at this stage.

The balance between noradrenaline, dopamine and 5-hydroxytryptamine concentrations in the brain will depend on the various enzyme systems involved in their synthesis and degradation as well as on the systems involved in their storage, uptake and release. It is not suggested that caeruloplasmin or a similar enzyme fulfils the functions of these systems but it may provide an additional sensitive control over the relative concentrations of noradrenaline, dopamine and 5-hydroxytryptamine in those areas of the brain where they act as neurotransmitters.

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