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Effects of Some Centrally Acting Drugs on Caeruloplasmin

B. C. BARRASS AND D. B. COULT

Ministry of Defence, Chemical Defence Establishment, Porton Down, Salisbury, Wiltshire (Great Britain)

Potent centrally acting drugs are of importance to the biological scientist not only because of their effects on mood, perception and behaviour, but also because they can be used as specific chemical probes for studying the CNS at the neurophysiological and neurobiochemical levels. Before such drugs can be fully and rationally exploited for this purpose it is necessary that their mode of action should be understood in some detail; at the present time the mode of action of none of the centrally active drugs is sufficiently well understood for them to be so used. In view of their potent peripheral anticholinergic activity the glycollates (Abood, 1968), probably exert their central effects by interfering with central cholinergic pathways (Brimblecombe and Green, 1968), but the way in which compounds such as LSD, mescaline and 2,5-dimethoxy-4-methyl amphetamine (DOM, STP) produce their characteristic central effects is less well understood. In view of the chemical similarity (Table I) between LSD, mescaline and DOM on the one hand and central neurotransmitters such as dopamine, noradrenaline (NA) and 5-hydroxytryptamine (5-HT, serotonin) on the other, a mode of action involving interference with central dopaminergic, noradrenergic or serotoninergic mechanisms seems generally likely (Giarman and Freedman, 1965; Freedman and Aghajanian, 1966). Certainly, in the case of LSD its known peripheral antagonism of 5-HT (Gaddum, 1953) has been extensively quoted as supporting the suggestion that its central effects are due to some kind of interference with an unspecified central serotoninergic system. However, it must be admitted 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, antagonism or destruction of natural neurotransmitters such as NA, dopamine and 5-HT have not so far proved entirely satisfactory.

In a search for a possible biochemical means of investigating the mode of action of LSD it was noted that the administration of this drug to animals resulted in an elevation of brain 5-HT levels and a depression of brain catecholamine levels (Barchas and Freedman, 1963; Koenig-Bersin *et al.*, 1970). It therefore seemed of interest to study the effects of LSD on an enzyme which could utilize both NA and 5-HT as substrates since it was considered that such an enzyme could be used as a 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 study in this way was the copper-containing oxidase caeruloplasmin which utilizes both NA and

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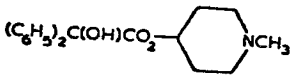
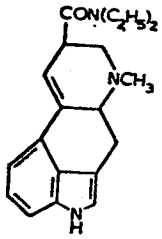
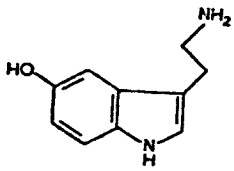
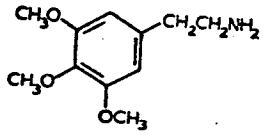
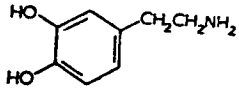
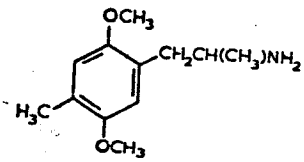
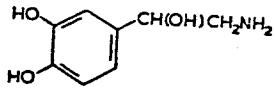
TABLE I

HALLUCINOGENIC DRUGS AND NEUROTRANSMITTERS

Hallucinogen	*Neurotransmitter*	*System Affected*
	$CH_3CO_2CH_2CH_2N^+(CH_3)_3$	Cholinergic?
		Serotoninergic?
		Dopaminergic/ Adrenergic?
		

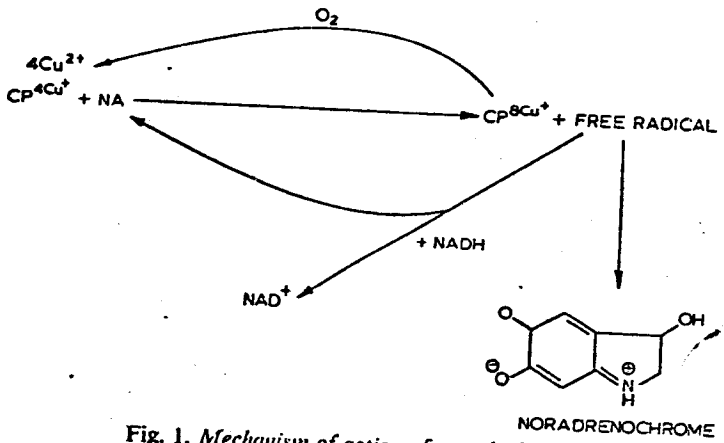


Fig. 1. *Mechanism of action of caeruloplasmin.*

$4Cu^{2+}$

CP^{4Cu^+}, Oxidised caeruloplasmin
CP^{8Cu^+}, Reduced caeruloplasmin
NA , Noradrenaline

5-HT as substrates in the cycle of reactions which are shown in Fig. 1. A similar scheme may be drawn using 5-HT as substrate.

The effects of various centrally active drugs on the caeruloplasmin-catalysed oxidation of NA and 5-HT were accordingly studied, the parameters monitored being the rates of formation of noradrenochrome and disappearance of NADH (both measured spectrophotometrically) and the rates of oxygen consumption and formation of NAD^+ (both being measured polarographically). The results obtained are summarised in Table II. Although all the compounds affected the caeruloplasmin-catalysed oxidation of both substrates, LSD had the most marked effect, in agreement with the fact that it is also the most potent hallucinogen of the 5 compounds shown in Table II.

TABLE II

EFFECTS OF SOME CENTRALLY ACTING INDOLES ON THE OXIDATION OF NORADRENALINE AND 5-HT BY CAERULOPLASMIN

Compound	*Action on oxidation of*		*Comments*
	Noradrenaline	*5-HT*	
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	

The inhibition of the enzymic oxidation of 5-HT by LSD is not unexpected in view of the chemical similarities of the two molecules, but the acceleration of the enzymic oxidation of NA was totally unexpected and raises some interesting problems regarding the mechanism of action of caeruloplasmin. Perhaps the most likely explanation for these effects of LSD is that the enzyme has two distinct binding sites, for NA and 5-HT respectively, but that these have the oxidative site in common. LSD could then be considered to interact with the 5-HT binding site in such a way as to inhibit binding of this particular substrate to the enzyme and either enhance the binding of NA or enhance the chemical reactivity of the oxidative site. This suggestion that caeruloplasmin has two distinct active sites is supported by the work of Curzon and Speyer (1968) who, using inorganic inhibitors such as azide and

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fluoride ions and unsymmetrical N,N-dimethyl *p*-phenylenediamine as the substrate, concluded that there were two 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 at this time but there is no evidence that allosteric effects are involved. Further work is needed to clarify this point.

In view of the above results obtained with LSD it was clearly desirable to extend these studies to other centrally active drugs such as glycollates, phenylalkylamines and indoles. The glycollates described by Abood in 1968 were without effect on the caeruloplasmin-catalysed oxidation of NA and 5-HT, an observation which is in accord with the suggestion that they exert their central effects by interfering with central cholinergic pathways.

The phenylalkylamines mescaline, 2,5-dimethoxy-4-methyl amphetamine (DOM) and 3,4-dimethoxyphenylethylamine had no effects on caeruloplasmin — they were neither substrates nor did they modify the oxidation of known substrates. In other studies on the substrate specificity of caeruloplasmin it was noted that, in addition to being a substrate, 3-hydroxy-4-methoxyphenylethylamine accelerated the oxidation of both noradrenaline and 5-HT. Since demethylated metabolites of mescaline have been isolated by Musacchio and Goldstein (1967) from rats given this drug, it is possible that it is these metabolites which are the chemical species actually responsible for the observed 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 compounds and their effects on the enzyme. This point requires further experimental study.

The indoles described by Brimblecombe *et al.* (1964) had no effect on caeruloplasmin, but again it is possible that with these compounds it is the hydroxylated metabolites which are the active species, as suggested by the above authors and also by Szara (1961). These metabolites would, however, be formed by hydroxylation of the aromatic ring whereas the hydroxy metabolites of compounds such as mescaline and DOM would be formed by the demethylation of one of the methoxyl groups which are such a characteristic structural feature of the centrally active phenylalkylamines.

From the studies so far described it seemed that LSD was almost unique amongst centrally acting compounds in the potency of its effects on the caeruloplasmin-catalysed oxidation of NA and 5-HT, and this has led to the suggestion that this enzyme, or one with similar properties, may be directly involved in the mode of action of LSD. It is known that the K_m values for NA and 5-HT are almost identical, suggesting that caeruloplasmin or an enzyme with similar properties 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 NA, dopamine and 5-HT is essential to normal mental function, then LSD could produce its central effects by disturbing the balance between these biogenic amines as a result of its interaction with caeruloplasmin or a similar enzyme; moreover, since LSD affects the

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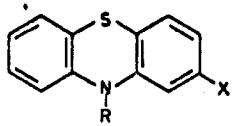
TABLE III

EFFECTS OF SOME PHENOTHIAZINES  ON THE OXIDATION OF NORADRENALINE AND 5-HT BY CAERULOPLASMIN

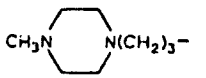
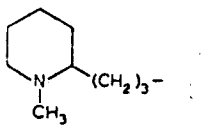
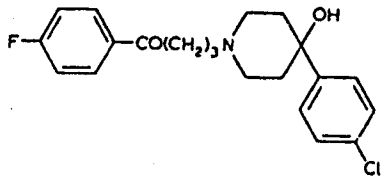
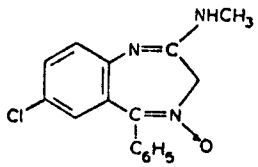
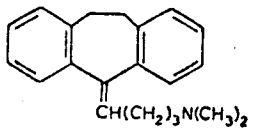
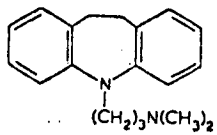
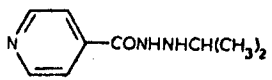
Compound		*Action on oxidation of*		*Comments*
R	*X*	*Noradrenaline*	*5-HT*	
$(CH_3)_2N(CH_2)_3$—	Cl	Catalysis. 200% at 10^{-3}M	Catalysis. 200% at 10^{-3}M	Tranquillizer (Chlorpromazine)
	CF_3	Catalysis. 200% at 2×10^{-4}M	Catalysis. 200% at 2×10^{-4}M	Tranquillizer (Trifluperazine)
$(CH_3)_2N(CH_2)_3$—	CF_3	Catalysis. 200% at 10^{-3}M	Catalysis. 200% at 10^{-3}M	Tranquillizer (Triflupromazine)
	SCH_3	Catalysis. 200% at 2×10^{-3}M	Catalysis. 200% at 2×10^{-3}M	Tranquillizer (Thioridazine)
$(CH_3)_2NCHCH_3$— 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)

TABLE IV

EFFECTS OF SOME ANTIDEPRESSANTS AND TRANQUILLIZERS ON THE CAERULOPLASMIN-CATALYSED OXIDATION OF NORADRENALINE AND 5-HT

Compound	*Effects on oxidation of*		*Comments*
	Noradrenaline	*5-HT*	
	Catalysis. 200% at 2×10^{-3}M	Catalysis. 200% at 2×10^{-3}M	Tranquillizer (Haloperidol)
	No effect	No effect	Minor tranquillizer (Chlordiazepoxide)
	Inhibition. 50% at 10^{-2}M	Inhibition. 50% at 10^{-2}M	Anti-depressant (Amitryptyline)
	Inhibition. 50% at 10^{-2}M	Inhibition. 50% at 10^{-2}M	Anti-depressant (Imipramine)
	No effect	No effect	Anti-depressant (Iproniazid)

oxidation of NA and 5-HT in opposite directions it should be more potent than a compound which affects the oxidation of only one of these substrates. Implicit in this suggestion is the assumption that caeruloplasmin or a similar enzyme is directly involved in the maintenance of normal mental function. Since these investigations had been concerned only with compounds capable of modifying normal behaviour it was decided, as a result of the above considerations, to include in this study some compounds which are known to modify abnormal behaviour, *i.e.*, compounds used clinically for the treatment of abnormal mental states. Some of these results are shown in Tables III and IV. The tranquillizers of the phenothiazine type accelerated the oxidation of both NA and 5-HT, whereas phenothiazines which lack useful tranquillizing properties (promethazine and diethazine) had no effect. It is interesting that Haloperidol, which is not a phenothiazine but which is a tranquillizer, also accelerated the oxidation of both 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 in anxiety states rather than psychotic states, had no effect on caeruloplasmin.

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

The above results tend to support the proposal that caeruloplasmin, or an enzyme with similar properties, plays an important role in the maintenance of normal mental function and that interference with this enzyme leads to the appearance of abnormal mental states. It is not possible at present, however, to draw any firm conclusions regarding the effects of a compound on caeruloplasmin and its potential clinical usefulness in the treatment of mental disorders. Such conclusions must await the results of further studies which the present work has shown to be desirable.

SUMMARY

1. The copper-containing oxidase caeruloplasmin was studied as a potential model for those receptors in the CNS with which certain centrally acting drugs must interact in order to produce their characteristic effects.

2. Lysergic acid diethylamide (LSD), and to a lesser degree ibogaine and 2-bromo-LSD, inhibited the enzymic oxidation of 5-HT but accelerated the oxidation of NA. Harmine and harmol inhibited the enzymic oxidation of both substrates.

3. Some phenylethylamines and anticholinergics with reported central activity had no effects on the enzymic oxidation of NA and 5-HT.

4. Certain of the drugs used in the treatment of mental illness affected the caeruloplasmin-catalysed oxidation of NA and 5-HT. Tranquillizers of the phenothiazine type, for example, accelerated the oxidation of both substrates, whilst anti-depressant drugs (other than monoamine oxidase inhibitors) inhibited the oxidation of both substrates.

5. The results have led to the tentative suggestion that caeruloplasmin, or an

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enzyme with similar properties, may be of importance in controlling the relative concentrations of NA and 5-HT in some areas of the brain. The relevance of this suggestion to the mode of action of LSD and other centrally acting drugs is discussed.

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DISCUSSION

PINDER: Is dopamine a substrate for caeruloplasmin? If so, does the enzyme play a role in mediating dysfunctions of brain copper metabolism such as in Wilson's disease, particularly since dopaminergic deficiency is involved in the symptomatically related condition of Parkinsonism?

COULT: Yes, I have talked a great deal about noradrenaline, but when I said noradrenaline I could equally have said dopamine. The two seem very much the same as far as this enzyme is concerned. In fact, dopamine has a slightly higher affinity than noradrenaline.

KING: Is there any drug which gives inhibition of noradenaline and catalysis of 5-HT?

COULT: We do not know of any such compound.

BRIMBLECOMBE: I find your evidence concerning the psychotherapeutic drugs—the tranquillizers and anti-depressants—rather more convincing than the evidence relating to the psychotomimetic drugs. For example, the evidence for the 6-hydroxylation of tryptamine *in vivo* is not very strong and we would like to know that the hydroxylated mescaline derivative that you mentioned is a psychotomimetic drug.

COULT: These compounds must be active at the site. The fact that 6-hydroxytryptamines have been put into animals does not mean that these are the compounds which reach the active sites.