Biochemical Pharmacology, Vol. 25, pp. 1877–1879. Pergamon Press, 1976. Printed in Great Britain.

# INTERACTION OF SOME NEUROLEPTIC AND ANTIDEPRESSIVE AGENTS WITH HUMAN COERULOPLASMIN

ROLF A. LØVSTAD

Institute of Medical Biochemistry, University of Oslo, Karl Johans gt. 47, Oslo 1, Norway

(*Received* 2 *October* 1975; *accepted* 26 *February* 1976)

**Abstract**—The coeruloplasmin oxidase activity towards some neuroleptic and antidepressive drugs was investigated. Prothipendyl, an azaphenothiazine, was found to have the same $V_{max}$-value as its phenothiazine analog, promazine. At lower substrate concentrations prothipendyl was more slowly oxidized than promazine, giving rise to a higher $K_m$-value. Imipramine, desipramine and opipramol were slowly oxidized by coeruloplasmin compared to promazine, while chlorprothixene, tiothixene, amitriptyline, protriptyline and haloperidol did not react at all. A central ring nitrogen atom seems to be essential for oxidase activity. Furthermore, the folded phenothiazine ring system is favourable for a rapid reaction. The best substrates, promazine and prothipendyl, activated the conversion of noradrenaline to noradrenochrome in the presence of coeruloplasmin.

Coeruloplasmin is a serum protein containing several copper atoms, namely the Type-1 ("blue") $Cu^{2+}$, the Type-2 ("non-blue") $Cu^{2+}$ and non-paramagnetic copper ions [1, 2]. In a previous communication it was shown that coeruloplasmin enzymatically oxidized phenothiazine derivatives to free radicals [3]. Further investigations showed that the coeruloplasmin activity decreased with increasing electron-withdrawing effect of the substituent in the 2-position of the phenothiazine ring, and that the enzyme had a higher affinity for phenothiazines with a piperazinyl-propyl side chain in the 10-position than for those with an aliphatic side chain [4]. In the present communication the coeruloplasmin activity towards tricyclic drugs, structurally related to the phenothiazines, was investigated in order to obtain further information on the substrate specificity of the enzyme.

## MATERIALS AND METHODS

*Materials.* Human coeruloplasmin was purchased from AB Kabi and crystallized according to the method of Deutsch [5]. The purified enzyme had an absorbance ratio, $A_{610}/A_{280}$, of 0.047. Enzyme concentrations were calculated from the 610 nm absorption ($\epsilon = 10{,}900\,M^{-1}\,cm^{-1}$) [5]. Promazine was obtained from AB Ferrosan, prothipendyl from Draco AB, imipramine from A/S Dumex, desipramine and opipramol from Ciba–Geigy, chlorprothixene and amitriptyline from Lundbeck & Co. A/S, tiothixene from Pfizer, protriptyline from Merck, Sharp & Dohme, haloperidol from AB Mekos, and noradrenaline and NADH from Sigma Chem. Co. All aqueous solutions were prepared in deionized, glass-distilled water.

*Measurement of enzyme activity.* The oxidase activity of coeruloplasmin towards the drugs studied was measured by adding NADH to the reaction solution and recording the change in absorption at 340 nm, due to the disappearance of NADH, which is spontaneously oxidized by the free radicals generated from the substrate [3]. The reaction mixture contained 0.25 mM NADH in 0.25 M sodium acetate buffer, pH 5.5 (30°). The reaction rate is independent of the NADH concentration. During the process of reoxidizing the enzyme copper, after reduction by substrate, oxygen is reduced to water by accepting four electrons. The rate of oxygen uptake was found to be exactly half of the oxidation rate of NADH, which is a two-electron donor.

The oxidase activity of coeruloplasmin towards noradrenaline was measured spectrophotometrically at 490 nm as the rate of aminochrome formation [6]. An iron-chelating agent, 1,10-phenanthroline, was added in order to eliminate the activating effect of trace iron ions on the enzymic oxidation of noradrenaline [7]. The reaction mixture contained 6 μM coeruloplasmin, 5 mM noradrenaline, 1.5 mM drug and 50 μM 1,10-phenanthroline in 0.25 M sodium acetate buffer, pH 5.5 (30°).

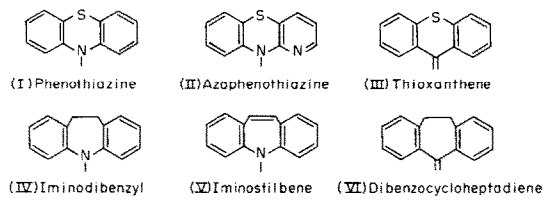
*Reduction of coeruloplasmin.* A mixture of drug and NADH was rapidly added to a solution of coeruloplasmin. The reduction of the blue colour of the enzyme by substrate was followed spectrophotometrically at 610 nm. The reaction mixture contained 44 μM coeruloplasmin, 1.33 mM drug and 0.25 mM NADH in 0.37 M sodium acetate buffer, pH 5.5 (30°). NADH alone did not reduce the blue colour of coeruloplasmin.

A Beckman DK-1 recording spectrophotometer, equipped with a thermo cell, was used in the kinetic experiments.

## RESULTS

The coeruloplasmin activity towards some tricyclic drugs is shown in Table 1. The compounds investigated had a three-carbon chain between the central ring and the nitrogen atom of the side chain. This has previously been shown to be essential for a rapid oxidation of the phenothiazine derivatives by coeruloplasmin [4]. Among the drugs investigated only promazine and prothipendyl were rapidly oxidized by

Table 1. Coeruloplasmin oxidase activity and effect of drugs on the oxidation of noradrenaline in the presence of coeruloplasmin




| Compound | Activity* (μM/min) | Activation of noradrenaline oxidation (% of control) |
|---|---|---|
| Promazine (I) | 95 | 430 |
| Prothipendyl (II) | 61 | 225 |
| Chlorprothixene (III) | 0 | 0 |
| Tiothixene (III) | 0 | 0 |
| Imipramine (IV) | 1 | 0 |
| Desipramine (IV) | 1 | 0 |
| Opipramol (V) | 1 | 0 |
| Amitriptyline (VI) | 0 | 0 |
| Protriptyline | 0 | 0 |
| Haloperidol | 0 | 0 |

* The reaction mixture contained 5 μM coeruloplasmin and 1 mM drug. For details see Materials and Methods.

coeruloplasmin. The enzyme activity towards imipramine, desipramine and opipramol was considerably lower (Table 1), while chlorprothixene, tiothixene, amitriptyline, protriptyline and haloperidol did not react at all.

In the Lineweaver–Burk plot in Fig. 1 the prothipendyl oxidase activity of coeruloplasmin is compared with its promazine oxidase activity. The maximum activity ($V_{max}$) was the same for both compounds, but at lower substrate concentrations promazine was more rapidly oxidized than its azaphenothiazine analog. The Michaelis constant ($K_m$) was 0.7 mM and 1.3 mM for promazine and prothipendyl, respectively. Figure 2 shows the reduction by promazine and prothipendyl of the 610 nm chromophore of coeruloplasmin, due to the Type-1 $Cu^{2+}$ [1, 2], which is directly involved in the catalytic process [8–10]. Promazine reduced the 610 nm chromophore faster than prothipendyl. A second-order rate constant of 0.2 $mM^{-1}\,sec^{-1}$ and 0.05 $mM^{-1}\,sec^{-1}$ was calculated for promazine and prothipendyl, respectively. The other drugs investigated did not reduce the blue colour of coeruloplasmin.

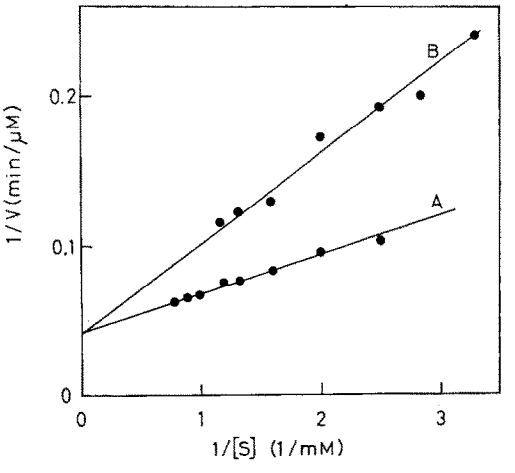



Fig. 1. Effect of promazine (A) and prothipendyl (B) (0.3–1.25 mM) on the rate of NADH oxidation in the presence of 1 μM coeruloplasmin.

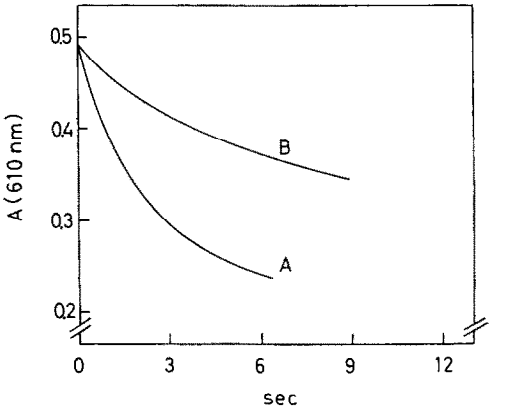



Fig. 2. Time course of the 610 nm absorbance change during reduction of coeruloplasmin by promazine (A) and prothipendyl (B).

Barrass and Coult [11] reported that some phenothiazine derivatives and haloperidol activated the coeruloplasmin-catalyzed oxidation of dopamine and noradrenaline to aminochrome. The present study shows that only prothipendyl, besides promazine, increased the rate of noradrenaline oxidation (Table 1). The observation of Barrass and Coult [11] that haloperidol activated this reaction could not be reproduced.

## DISCUSSION

The present results suggest that there is a difference in the azaphenothiazine and phenothiazine interac-

tion with coeruloplasmin. Under saturated enzyme conditions the rate of product formation from prothipendyl and promazine is the same (Fig. 1). The lower oxidation rate of prothipendyl at lower substrate concentrations, giving rise to a higher $K_m$-value, suggests that the enzyme has a lower affinity for this compound or that prothipendyl has a lower electron donor ability than promazine. The observation that prothipendyl reacts more slowly with the enzyme Type-1 $Cu^{2+}$ than promazine (Fig. 2) also indicates a slower formation of the enzyme–substrate (product) complex in this case. The good electron donor ability of the phenothiazines is essentially due to the ring nitrogen atom as reported by several investigators [12, 13]. Thioxanthene, having a carbon atom instead of the central ring nitrogen atom, was found to display lower electron donor ability than phenothiazine [13], and chlorprothixene considerably lower electron donor ability than chlorpromazine [14]. Chlorprothixene and tiothixene did not react with coeruloplasmin (Table 1), indicating that the nitrogen atom of the central ring is essential for enzyme activity.

Iminodibenzyl and iminostilbene, having a seven-membered central ring and a ring nitrogen atom (Table 1) possess a remarkable electron donor ability [13]. However, the iminodibenzyl derivatives, imipramine and desipramine, and opipramol, an iminostilbene derivative, were very slowly oxidized by coeruloplasmin compared to promazine (Table 1), suggesting that the structure of the tricyclic ring system is of importance for oxidase activity. Opipramol has a planar ring system due to the carbon–carbon double bond of the central ring [15], while X-ray analysis of imipramine reveals a complex folding of the ring system [16]. The phenothiazine derivatives are folded along the central —S—N— axis with an angle of approximately 140° between the planes of the benzene rings [17], a conformation which seems favourable for a rapid reaction with coeruloplasmin. It has been suggested that the sulphur atom may be confering flexibility on the molecule [12], and the possibility therefore exists that the flexibility of the ring system may result in a better binding of the tricyclic drug to the active site on coeruloplasmin.

The amitriptyline ring system differs from iminodibenzyl, and the protriptyline ring system from iminostilbene in that the ring nitrogen is replaced with a carbon atom. These compounds do not react with coeruloplasmin (Table 1), supporting the assumption that the ring nitrogen is essential for enzyme activity. Haloperidol, a good electron donor [14], was also tested, but it was not oxidized by coeruloplasmin. A correlation between the coeruloplasmin oxidase activity and the electron donor ability of the drugs could not be established.

It has previously been suggested that the activating effect of phenothiazines on the coeruloplasmin-catalyzed oxidation of catecholamines, reported by Barrass and Coult [11], was due to a rapid oxidation of catecholamines by the phenothiazine radicals generated by the action of coeruloplasmin on the phenothiazine derivatives [3]. The best substrates were found to be the best activators [4]. The present results are in accordance with this observation. Only promazine and prothipendyl, being good substrates, activated the oxidation of noradrenaline to aminochrome. In this connection it is interesting that schizophrenic patients on prolonged phenothiazine therapy have melanin deposits in several organs [18, 19]. A formation in the organism of phenothiazine radicals [20], rapidly oxidizing dopa and other catecholamines [3, 4], could possibly account for this. Phenothiazine treatment can also result in a Parkinsonian condition, which is characterized by a decrease in the dopaminergic activity in the nigrostriatal–pallidal complex [21].

*Acknowledgements*—The author thanks Professor O. Walaas and Dosent E. Walaas for their help and interest, AB Ferrosan, Draco AB, Ciba-Geigy, A/S Dumex, Lundbeck & Co. A/S, Pfizer, Merck, Sharp & Dohme and AB Mekos for the supply of drugs. This work was supported by grant from Anders Jahres Fond.

## REFERENCES

1. R. Malkin and B. G. Malmström, in *Advances in Enzymology* **33,** (Ed. F. F. Nord) p. 177. Interscience Publishers, New York (1970).
2. L-E. Andreasson and T. Vänngård, *Biochim. biophys. Acta* **200,** 247 (1970).
3. R. A. Løvstad, *Biochem. Pharmac.* **23,** 1045 (1974).
4. R. A. Løvstad, *Biochem. Pharmac.* **24,** 475 (1975).
5. H. F. Deutsch, *Archs Biochem. Biophys.* **89,** 225 (1960).
6. R. A. Løvstad, *Acta chem. scand.* **25,** 3144 (1971).
7. J. A. McDermott, C. T. Huber, S. Osaki and E. Frieden, *Biochim. biophys. Acta* **151,** 541 (1968).
8. L. Broman, B. G. Malmström, R. Aasa and T. Vänngård, *Biochim. biophys. Acta* **75,** 365 (1963).
9. S. Osaki and O. Walaas, *J. biol. Chem.* **242,** 2653 (1967).
10. E. Walaas, R. A. Løvstad and O. Walaas, *Archs Biochem. Biophys.* **121,** 480 (1967).
11. B. C. Barrass and D. B. Coult, *Biochem. Pharmac.* **21,** 677 (1972).
12. J. E. Bloor, B. R. Gilson, R. J. Haas and C. L. Zirkle, *J. med. Chem.* **13,** 922 (1970).
13. M. J. Mercier and P. A. Dumont, *J. Pharm. Pharmac.* **24,** 706 (1972).
14. M. Saucin and A. Van de Vorst, *Biochem. Pharmac.* **21,** 2673 (1972).
15. I. Sjöholm and T. Sjödin, *Biochem. Pharmac.* **21,** 3041 (1972).
16. M. L. Post, O. Kennard and A. S. Horn, *Nature* **252,** 493 (1974).
17. J. L. Coubeils and B. Pullman, *Theor. chim. Acta, Berlin* **24,** 35 (1972).
18. A. C. Greiner and G. A. Nicolson, *Lancet* **II,** 1165 (1965).
19. G. A. Nicolson, A. C. Greiner, W. J. G. McFarlane and R. A. Baker, *Lancet* **I,** 344 (1966).
20. D. C. Borg and G. C. Cotzias, *Proc. natn. Acad. Sci. U.S.A.* **48,** 643 (1962).
21. O. Hornykiewicz, *Br. med. Bull.* **29,** 172 (1973).