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HISTAMINE HAS NO INFLUENCE ON THE OXIDATIVE PROPERTIES OF CERULOPLASMIN

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Abstract—Histamine dihydrochloride increases ceruloplasmin-catalyzed reactions with *p*-phenylenediamine and *o*-dianisidine. This effect is entirely due to the influence of chloride ions; histamine itself does not alter ceruloplasmin activity. The effect of chloride and phosphate ions on ceruloplasmin activity is reported, and the significance of these findings relative to previous assays for amine oxidase is discussed. In addition, the histamine H_2-receptor antagonists: burimamide and metiamide were found to cause disappearance of colour formed during *o*-dianisidine oxidation; this effect probably depends on the thiourea residue.

o-Dianisidine (*o*-DA) and *p*-phenylenediamine (*p*-PD) are substrates for ceruloplasmin (Cp) [1, 2, 3] and Cp oxidising activity may be measured against these diamines [1, 3]. Histamine dihydrochloride increases Cp activity [2, 4]. The purpose of this paper is to show that this increase is due to the anion and not the base as previously thought [4]. It has been shown that Cp may interfere with determination of histaminase by the *o*-DA test [2]. Accordingly, additional experiments were performed in phosphate buffer pH 7.0 to evaluate the influence of this medium (commonly used in amine oxidase determinations) on Cp-catalyzed reactions. The interference of two H_2-receptor antagonists with the estimation of Cp has also been studied.

MATERIALS AND METHODS

Purified human ceruloplasmin (5% solution in 0.15 M NaCl) was purchased from WSS, Warsaw, Poland; *p*-phenylenediamine dihydrochloride-*p*-PD (Merck); *o*-dianisidine (3,3′-dimethoxybenzidine BDH); burimamide and metiamide (Smith, Kline and French Labs., England); histamine dihydrochloride and histamine diphosphate (Sigma); histamine base (Fluka).

When Cp oxidase activity was measured against *o*-DA, the substrate concentration was 8.19×10^{-4} M, in acetate buffer pH 5.5, total volume 2 ml. The reaction was stopped by addition of 2 ml 9.8 N HCl; this produced a colour change from brownish to pink (absorbance maximum at 535 nm [5]). In the reaction with *p*-PD as the Cp substrate (final concn 5×10^{-5} M in acetate buffer pH 5.5) the reaction was ended by addition of sodium azide. The coloured product was measured at 530 nm [3].

RESULTS

Addition of histamine dihydrochloride to the reaction mixture enhances the oxidative activities of Cp against *o*-DA and *p*-PD. The effect is much more evident if the reaction is carried out in phosphate buffer at pH 7.0 than in acetate buffer at pH 5.5.

This enhancing action was also seen when histamine dihydrochloride was replaced by an equivalent amount of sodium chloride (Fig. 1). Phosphate ions on the other hand depressed Cp activity when measured in acetate buffer (Fig. 2). High concentrations of chloride ions ($>10^{-2}$ M) also had a depressant action in this medium.

Because of Bozhkov's report of an effect of histamine on Cp activity [4], and our own previous results [2] it was necessary to estimate Cp in the presence of H_2-receptor antagonists. In the presence of burimamide and metiamide *o*-DA oxidation by Cp was decreased. The experiments reported in Fig. 3 show that

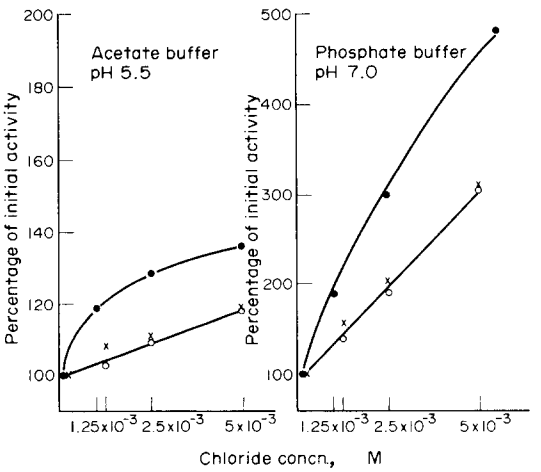


Fig. 1. Influence of histamine dihydrochloride and NaCl (expressed in final concentrations) on the *o*-DA (●——●) and *p*-PD (○——○) oxidations. General conditions of incubation: *o*-DA—8.19×10^{-4}M and *p*-PD × 2 HCl—5×10^{-4} M in final concentrations. Two-ml mixtures in acetate and phosphate buffers contain 33 μg and 150 μg ceruloplasmin respectively. Each point represents mean value from four samples. Results for NaCl in place of histamine dihydrochloride in *p*-PD oxidations (×).

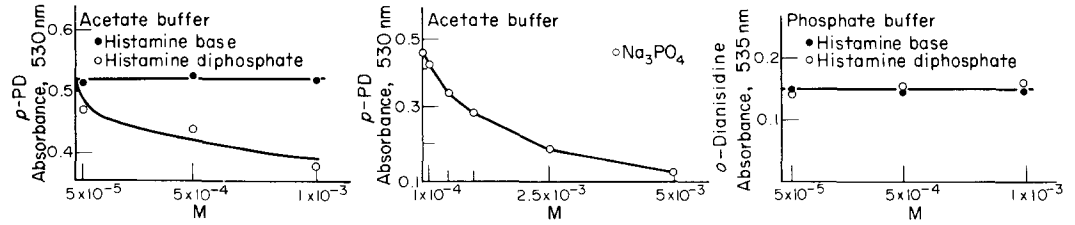


Fig. 2. The effect of histamine diphosphate, phosphate ions, and histamine base (expressed in final concentrations) on ceruloplasmin activity. Substrate and enzyme concentrations are described on Fig. 1. Each point is the mean of four determinations.

this effect was not due to a change of enzyme activity, but due to the instability of the chromophore formed during the reaction. Thus, addition of burimamide or metiamide to the acidified oxidation product of *o*-DA caused the slow disappearance of the colour.

Thiourea, but not imidazole, also caused the colour to fade and as this group is common to the side chain of both H_2-antagonists it is implicated in the interference produced by these antagonists.

DISCUSSION

Chloride ions in low concentrations increase Cp activity; higher concentrations inhibit [1, 6, 7]. Activation is especially evident at neutral pH and in phosphate buffer (Fig. 1). Phosphate anions themselves inhibit the oxidative activity of Cp [6] and (Fig. 2).

This fact may explain why histamine diphosphate inhibits Cp activity in acetate buffer. Addition of chloride ions (in form of histamine dihydrochloride) to enzyme inhibited by phosphate ions, reversed this inhibition. A similar effect of chloride ions on azide-inhibited Cp was described by Curzon [7]. Our data (Figs. 1 and 2) suggest that the influence of histamine on Cp activity is mainly related to the effects of particular anions but not to histamine itself. Histamine is neither a substrate for Cp [2] nor a regulator of its activity. We presume that our results also have a methodological significance. Several methods have been described [5, 8, 9, 10] in which *o*-DA was used to assay activity of several amine oxidases (including histaminase), not only in tissues but also in plasma; Cp is a normal constituent of plasma. Thus while a blank contains only plasma (amine oxidase and Cp together), *o*-DA and peroxidase, in the sample proper histamine (or any other amine) is added, usually as the hydrochloride. The chloride ions increase *o*-DA oxidation by Cp relative to the blanks. The elevated *o*-DA oxidation might be mistaken for amine oxidase activity and treated as oxidative deamination of histamine (or other amines).

Hampton *et al.* [11] reported the existence of histaminase activity of Cp. Their results may be

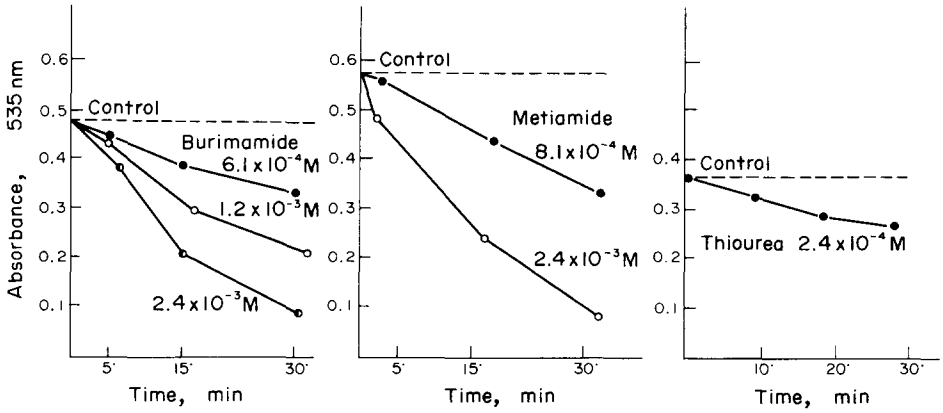
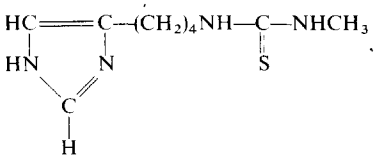
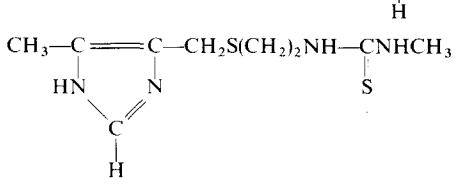
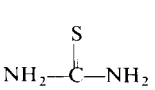


Fig. 3. The influence of burimamide  , metiamide  and thiourea  on the disappearance of the chromophore of *o*-DA oxidation. Different initial values are connected with different samples of enzyme.

explained by such a Cp-catalyzed reaction of *o*-DA which is more rapid in the presence of chloride ions (histamine dihydrochloride) especially in phosphate buffer and at pH 7.0.

Our data also show that false Cp activity values might be obtained using *o*-DA if burimamide, metiamide or thiourea are present in the incubation mixture (or in plasma). Amine oxidases determinations (MAO or DAO) are also subject to the same interference for the final product of *o*-DA oxidation is the same. Thus their estimation by an *o*-DA test may give the unreliable results in the presence of thiourea compounds e.g. those used in the treatment of thyroid diseases.

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