Ceruloplasmin impairs endothelium-dependent relaxation of rabbit aorta

MAURIZIO CAPPPELLI-BIGAZZI,1 GIUSEPPE AMBROSIO,2 GIOVANNI MUSCI,3 CARMINE BATTAGLIA,1 MARIA CARMELE BONACCORSI DI PATTI,4 PAOLO GOLINO,1 MASSIMO RAGNI,1 MASSIMO CHIARIELLO,1 AND LILIA CALABRESE5

1Division of Cardiology, Second School of Medicine, University of Naples, Naples 80131;
2Division of Cardiology, School of Medicine, University of Perugia, Perugia 06100; 3Department of Organic and Biological Chemistry, University of Messina, Messina 98166; 4Department of Biochemical Sciences and Consiglio Nazionale delle Ricerche Center of Molecular Biology, University of Rome, La Sapienza, Rome 00185; and 5Department of Biology, University of Rome, Roma Tre, Rome 00154, Italy

Cappelli-Bigazzi, Maurizio, Giuseppe Ambrosio, Giovanni Musci, Carmine Battaglia, Maria Carmela Bonaccorsi di Patti, Paolo Golino, Massimo Ragni, Massimo Chiariello, and Lilia Calabrese. Ceruloplasmin impairs endothelium-dependent relaxation of rabbit aorta. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H2843–H2849, 1997.—This study evaluated the effects of ceruloplasmin, the copper-containing blue oxidase of vertebrate plasma, on the relaxation of rabbit aortic rings after endothelial release of nitric oxide (NO). Ceruloplasmin at physiological, i.e., micromolar, concentrations inhibited relaxation of rabbit aorta induced by endothelium-dependent agonists like acetylcholine or ADP, whereas it was ineffective toward vasodilation due to direct stimulation of smooth muscle cells by nitroglycerin. The effect was reversible and specific for native, fully metalated ceruloplasmin, since relaxation was not impaired by the heat-treated or metal-depleted derivatives. A trapping mechanism, involving a direct interaction of NO or other NO-containing species (like nitrosothiols and iron-dinitrosyls) with the copper sites and/or with the free thiol of ceruloplasmin, could be safely excluded on the basis of spectroscopic and chemical analyses of the protein exposed to authentic NO, nitrosothiols, or iron-dinitrosyls. The data presented in this paper constitute the first evidence of impairment of the endothelium-dependent vasodilation by a plasma protein and may shed some light on the still uncertain physiological role of ceruloplasmin.

Nitric oxide; copper

Nitric oxide (NO) is synthesized in many cellular types, and it serves as an important intercellular mediator in the vasculature, central nervous system, kidney, and endocrine system (21). In the vascular compartment, NO produced by the endothelial NO synthase (NOS) diffuses into neighboring smooth muscle cells, where it induces an increase of guanosine 3',5'-cyclic monophosphate levels leading to altered calcium mobilization and to vessel relaxation (28, 39). For this reason, NO has been identified as the endothelium-derived relaxing factor (EDRF) (8) and plays a key role in the complex regulation of local and systemic vascular resistance, distribution of blood flow and oxygen delivery, and eventually regulation of arterial pressure (40).

Little is known about the involvement of high-molecular-weight plasma factors in the modulation of the endothelial NOS activity apart from a well-established role for thrombin as a vasodilating agent. Recently, it has been reported that immunoglobulins can inhibit the thrombin-induced NO production by endothelial cells (35). On the other hand, a specific role is emerging for some blood proteins in the stabilization and targeting of NO to specific effectors. In this respect, it has been suggested that albumin acts in the plasma as a reversible trap for NO through S-nitrosylation at its free cysteine and that the resulting stable albumin-NO complex, in conjunction with the extracellular pool of S-nitroso compounds (which also include S-nitrosocysteine and S-nitrosothiol groups), serves as a source of NO, prolonging its half-life in the blood and buffering its free concentration, and it eventually leads to vasorelaxation (14, 34, 36). Moreover, the existence of S-nitrosylated hemoglobin possessing vasorelaxing properties has been demonstrated in vivo and is related to a dynamic cycle involving the uptake of NO in the lung and its release during arterial-venous transit (13).

Besides nitrosothiols, it has been demonstrated that NO can be physiologically trapped within adducts with thiols and iron. A role for these paramagnetic iron dinitrosyl complexes (which have been shown to form in a number of different cell types, including agonist-stimulated endothelial cells) has been suggested in the control of blood vessel tone through transmembrane transport of NO (24).

Ceruloplasmin is the major extracellular copper-containing protein of vertebrates, bearing six copper atoms bound at multiple sites and able to interact with a number of ligands, including oxygen, azide, halides, and NO. The protein circulates at micromolar concentrations in the plasma, and although its existence was recognized several years ago, its physiological role is still under debate. It is certainly involved in iron metabolism, since it possesses a ferroxidase activity (29), and individuals lacking a functional ceruloplasmin gene have an impaired iron metabolism (11). The alternative function proposed for ceruloplasmin, that it acts as a copper-transport protein (3), cannot however be discounted yet. In fact, there is accumulating evidence of a multifunctional role for this protein. To unravel new roles of ceruloplasmin in the plasma, we investigated the effect of ceruloplasmin on the endothelium-dependent relaxation of rabbit aorta.
MATERIALS AND METHODS

Drugs and chemicals. The following pharmacological agents were used: prostaglandin F$_2$-a$_a$ (PGF$_2$-a$_a$)-dinoprost thromboxane (Upjohn, Kalamazoo, MI); nitroglycerin (Simes, Milan, Italy); and acetylcholine chloride, indomethacin, and ADP (Sigma Chemical, St. Louis, MO). Sepharose 4B and QAE-Sephadex A-50 were from Pharmacia, Uppsala, Sweden. Chloroethyliamine was from Carlo Erba, Milan, Italy and was recrystallized before use as previously described (2). Metals were removed from the buffers with Chelex (Bio-Rad, Richmond, CA) before use. All other reagents were of analytic grade and were used without further purification. Indomethacin was dissolved in 50% ethanol (final bath concentration of ethanol was 40 µM). All other drugs were dissolved in double-distilled water to an appropriate concentration such that ≤0.1 ml of each solution was added to the organ bath for each concentration.

Preparation of ceruloplasmin. Ceruloplasmin was purified from sheep, rabbit, or human plasma by single passage of plasma on Sepharose 4B derivatized with chloroethyliamine, as previously described (2). Passage on quaternary amino ethyl resin was used to concentrate the protein and to improve the purity of the sample. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis (17) revealed a high degree of purity (ranging from 95 to 99% in different samples) and showed that over 90% of the protein was present as the unfragmented 130,000-Da component. The physicochemical parameters (copper content, oxidase activity) and the spectroscopic properties [optical, electron paramagnetic resonance (EPR)] of the purified proteins were in line with those already published (2, 25). In particular, different preparations had a copper stoichiometry ranging from 5.1 to 5.5 Cu/molecule. The stoichiometry of each preparation was not affected by treatment of the protein with Chelex.

Copper-depleted ceruloplasmin (apoceruloplasmin) was prepared by incubating the native protein with 50 molar excess diethylidithiocarbamate (DDC) for 1 h at room temperature, centrifuging at 25,000 g to remove the insoluble copper-DDC complex, and then using exhaustive dialysis against 50 mM phosphate buffer, pH 7, to eliminate unreacted DDC. Heat-inactivated ceruloplasmin was obtained by heating the protein at 70°C for 90 min. Inactivation was assessed by irreversible loss of the optical absorption at 610 nm. Both apo- and heat-treated ceruloplasmin had a residual oxidase activity of ~10% with respect to the native protein. The residual activity was consistent with the presence of ~10% unremoved copper in apoceruloplasmin and of ~10% of the original absorption at 610 nm in the heat-treated protein.

Optical spectra were recorded on a Perkin-Elmer 330 spectrometer equipped with a Haake Mod G temperature controller. X-band EPR spectra were run at liquid nitrogen temperature on a Varian E-9 spectrometer and at room temperature on a Bruker ESP 300.

Incubations of ceruloplasmin with NO were carried out as previously described (27). To study the formation of nitrosothiols, we incubated sheep ceruloplasmin or bovine serum albumin (BSA) under increasing pressures of NO in the presence of ~0.05 atm of oxygen. Nitrites and nitrosothiols were quantitated after Tracey (38) and Saville (33), respectively. Free protein thiols were measured with 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) after Eillman (5). Dinitrosyl iron cysteine (DNIC 1:20) and DNIC-BSA were prepared according to Boese et al. (1).

Experimental model. Experiments were performed on thoracic aortas taken from male New Zealand White rabbits (1.5–2 kg) anesthetized with ketamine. Immediately after the rabbits were killed, the thoracic aortas were dissected free and placed in cold modified Krebs-Ringer bicarbonate solution (mM composition: 118.3 NaCl, 4.7 KCl, 2.5 CaCl$_2$, 1.2 MgSO$_4$, 1.2 KH$_2$PO$_4$, 25.0 NaHCO$_3$, 0.026 calcium-EDTA, and 11.1 glucose). They were cleared of connective tissue with care taken not to damage the intimal surface, and 4- to 6-mm-long rings were obtained.

Fine stainless steel wire clips were inserted through the lumen of the rings, allowing them to be suspended for recording of isometric tension. Rings were mounted in a jacketed organ bath filled with 25 ml of Krebs-Ringer solution maintained at 37°C and equilibrated with 95% O$_2$/5% CO$_2$, pH 7.4. Tension was measured by a force transducer, and changes in isometric force were recorded with a Gould 2400S direct-writing recorder coupled to bridge amplifier (Gould Instruments, Cleveland, OH). Rings were progressively stretched until the contractile response evoked by 100 mM KCl was maximal. The vessels were left at this length throughout the study.

Experimental protocol. When the optimal point of the length-tension curve of each vascular ring was achieved, indomethacin (10 µM) was added to the organ chamber and left throughout the experiment. To study endothelium-dependent and independent relaxation, we contracted rings with 2 µM PGF$_2$-a$_a$. A cumulative concentration-response curve to acetylcholine (10$^{-9}$–10$^{-5}$ M) was obtained in all vessels studied to assure endothelium integrity. After the first concentration-response curve, acetylcholine was washed out, and rings were recontracted with 2 µM PGF$_2$-a$_a$ and divided into three groups, each of six vessels, receiving different concentrations of purified ceruloplasmin (1, 3, and 10 µM). After addition of ceruloplasmin, a second dose-response curve to acetylcholine was performed. In three experiments, relaxation to acetylcholine was also repeated 30 min after washout of ceruloplasmin. In another group of six vessels, the effects of the highest concentration of ceruloplasmin (10 µM) on the endothelium-dependent relaxation to ADP were tested. Endothelium-independent vasodilation to nitroglycerin was studied in six additional aortic rings before and after incubation with 10 µM ceruloplasmin. In two additional groups of six vessels each, endothelium-dependent relaxation to acetylcholine was evaluated before and after incubation with 10 µM heat-treated or copper-free ceruloplasmin.

Data analysis and statistics. Data are expressed as means ± SE. Relaxation to acetylcholine, nitroglycerin, and ADP is expressed as the percent change relative to the constriction produced by PGF$_2$-a$_a$. The effects of ceruloplasmin on the dose-response curve to the various agents were tested for significance using analysis of variance with a design of repeated measures.

RESULTS

Figure 1 shows the profile of the endothelium-dependent relaxation of the aortic vessel to increasing concentrations of acetylcholine in the presence of varying amounts (1–10 µM) of sheep ceruloplasmin. The response of the vessel to acetylcholine was found to be progressively impaired by increasing concentrations of ceruloplasmin. Maximal relaxation was 87.7 ± 4.6% of baseline in control conditions and was already significantly (P < 0.01) reduced at 3 µM ceruloplasmin (60.5 ± 6.5%). Maximal relaxation observed in the presence of 10 µM ceruloplasmin (42.3 ± 7.29%) corresponded to over 50% inhibition. Computer extrapol-
tion of the effect of ceruloplasmin at infinite concentration of the protein yielded a maximum inhibitory effect of \(-75\%\). The effect was specifically due to ceruloplasmin, since different preparations of the protein with different degrees of purity (i.e., 95–99\%) gave essentially the same results, and was independent of the slight variations in the copper stoichiometry between different batches of ceruloplasmin. Treatment of ceruloplasmin with Chelex to remove loosely bound metals did not affect the ability of the protein to impair relaxation. Substitution of human or rabbit ceruloplasmin for the sheep protein resulted in comparable effects on the acetylcholine-induced relaxation of the vessel.

To test for the reversibility of the inhibitory effect of ceruloplasmin, rings treated with acetylcholine in the presence of 10 \(\mu\)M ceruloplasmin were extensively washed with buffer and allowed to reequilibrate for 30 min in the chamber bath. Maximal relaxation of these rings to acetylcholine was \(81.6 \pm 6.9\%\) (\(P = \text{not significant vs. control relaxation}\)), indicating that the effect of ceruloplasmin was reversible.

The effect of ceruloplasmin was also tested on the endothelium-dependent relaxation to a different agonist, namely ADP. The corresponding curves are shown in Fig. 2. In this case, maximal relaxation was \(83.4 \pm 4.9\%\) of PGF\(_2\alpha\) in control conditions, and it was reduced to \(49.2 \pm 5.4\%\) in the presence of 10 \(\mu\)M ceruloplasmin (\(P < 0.01\)). In contrast, relaxation to nitroglycerin, an endothelium-independent vasodilator, was not affected by 10 \(\mu\)M ceruloplasmin (Fig. 3). These data suggest that the effects of ceruloplasmin were specific for endothelium-mediated vasodilation, whereas direct vascular smooth muscle relaxation was not affected by the protein.

Two functionally inactive derivatives of ceruloplasmin, the heat-denatured and the copper-depleted proteins, were tested for their ability to inhibit the endothelium-dependent relaxation to acetylcholine. As shown in Fig. 4, neither derivative was effective, suggesting that the molecular architecture and the occupancy of the native sites by copper atoms in the protein must be preserved for the inhibition to occur. The slight inhibition observed with both heat-treated and apoceruloplasmin could be ascribed to the fraction of native holoprotein present in the samples (see MATERIALS AND METHODS).

It is known that ceruloplasmin can bind NO when exposed to high tensions of the authentic gas (10, 27). The binding occurs at the multiple copper sites of the protein and induces the variation of the optical absor-
bance at 610 nm for the blue copper sites, and in the 300- to 500-nm region for the other copper sites, the trinuclear cluster constituting the oxygen binding site. Figure 5 reports the change in the absorbance of sheep ceruloplasmin at some representative wavelengths in the 300- to 500-nm region as a function of NO pressure. We calculated the apparent affinity of ceruloplasmin for NO by fitting data at each wavelength to a single hyperbola. As shown Fig. 5, inset, the resulting values of $P_{50}$ (NO pressure at which the optical properties of ceruloplasmin change to a 50% extent) depended on the wavelength of observation. This would suggest that NO interacts at multiple sites (or class of sites) on sheep ceruloplasmin with different affinities. More importantly, however, was the fact that $P_{50}$ was never $<0.15$ atm NO, a pressure leading under our temperature and ionic strength conditions to $0.2$ mM dissolved NO.

Thiols can be reactive toward NO, forming nitrosothiols (RSNO) in the presence of oxygen (15). This has been shown to occur not only with low-molecular-weight thiols (cysteine, glutathione) but also with free cysteines in proteins like albumin (36) and hemoglobin (13), and a biological role for these latter adducts has been proposed. To test whether the single free cysteine of sheep ceruloplasmin could be S-nitrosylated, we first exposed ceruloplasmin to increasing pressures of NO in the presence of a small amount of oxygen. At variance with BSA, where $0.7$ RSNO per molecule formed on this treatment, no nitrosothiol formation could be detected with ceruloplasmin even at the highest NO pressure tested (1.5 atm). The formation of nitrosothiols on ceruloplasmin by transnitrosation reactions between the protein and low- or high-molecular-weight RSNO was also shown by the following experiment not to occur. Sheep ceruloplasmin was incubated with a large excess (10- to 100-fold) of either S-nitrosogluthathione (GS-NO) or S-nitroso-BSA (BSA-NO). At different times ranging from 5 min to 3 h, ceruloplasmin was quickly separated from the incubation mixture by the same method used for its purification, and the amount of RSNO was chemically assessed on both the protein and the bulk nitrosothiol. No RSNO could be detected on the protein at any time with any nitrosothiol, whereas determination of RSNO on bulk nitrosothiol gave indistinguishable values in samples incubated with and without ceruloplasmin. The low reactivity of the single free cysteine of ceruloplasmin is consistent with the observation that its rate of reaction with DTNB in the Ellman reaction was about 20-fold lower than that of BSA under comparable conditions (data not shown).

Previous evidence has been presented on the involvement of iron-dinitrosyls, complexes formed by NO, iron, and a thiol, in the NO-mediated vasorelaxation (23). To assess whether ceruloplasmin interacted with iron-dinitrosyl complexes, we first investigated whether paramagnetic DNIC 1:20 could form an adduct with sheep ceruloplasmin, analogously to what has been shown for albumin (1). EPR spectroscopy at room temperature was used in this respect to show that the line shape of DNIC 1:20 (which changes on binding of the complex to BSA) (Fig. 6, spectra A and B) was totally unaffected by ceruloplasmin (Fig. 6, spectrum C). The kinetics of NO release from the complex were then evaluated by measuring the formation of nitrates, and it was found that also in this respect the presence of ceruloplasmin was irrelevant. Finally, the EPR line shape of the high-molecular-weight complex BSA-DNIC was found to be insensitive to ceruloplasmin (Fig. 6, spectra B and D).

\[ \Delta \text{OD, change in optical density.} \]

Fig. 5. Variation of the absorbance at selected wavelengths in the optical spectrum of sheep ceruloplasmin incubated under increasing pressures of nitric oxide (NO). $\Delta \text{OD}$, change in optical density. Inset: variation of $P_{50}$ (the value of NO pressure at which optical properties of ceruloplasmin change to a 50% extent) with wavelength, obtained by fitting data at each wavelength to a single hyperbola.

Fig. 6. Room temperature electron paramagnetic resonance spectra of 100 µM DNIC 1:20 as such (A) and after addition of 150 µM bovine serum albumin (B), 150 µM sheep ceruloplasmin (C) or 150 µM bovine serum albumin and 150 µM sheep ceruloplasmin (D). Samples were in 30 mM N-2-hydroxyethylpiperazine-N' -2-ethanesulfonic acid buffer, pH 7.4. Spectrometer settings were the following: frequency, 9.79 GHz; center field, 0.344 T, modulation amplitude, 0.05 mT (spectra A and C) or 0.5 mT (spectra B and D).
DISCUSSION

In the present study, we present evidence that ceruloplasmin, a circulating copper protein, efficiently impairs in vitro relaxation of the aorta to endothelium-dependent vasodilators. The effect was found to be reversible and specific for the endothelial functions, since the protein did not interfere with direct vascular smooth muscle relaxation stimulated by nitroglycerin. The presence of copper bound to ceruloplasmin was a requisite for the inhibitory effect of the protein, as demonstrated by the results with apoceruloplasmin. However, the inefficacy of the heat-denatured sample, where copper is still present, indicates that the mere presence of copper does not per se warrant the inhibitory properties of the protein and therefore that ceruloplasmin has to be in a native state to impair relaxation. Note that both removal of copper and protein denaturation affect the overall conformation of ceruloplasmin (26), which explains why both apo- and heat-treated ceruloplasmins were ineffective.

Different mechanisms can explain the observed phenomenon. The hypothesis that ceruloplasmin simply acts as a trap for NO, thereby preventing the messenger from activating guanylate cyclase within the smooth muscle cell, is unlikely, because the affinity of ceruloplasmin for NO, as monitored by the spectral changes of the protein, cannot account for a significant trapping of NO under the conditions of the relaxation experiments. As a matter of fact, because constitutive NOS, including endothelial NOS, releases NO at micromolar concentrations at the most, only a very small fraction of liberated NO would be trapped by micromolar ceruloplasmin. Even when we consider that in our experimental conditions there are many orders of magnitude more moles of ceruloplasmin constantly flowing past the immediate vicinity of an NO-producing cell, the kinetics of the interaction, which we had shown to have a half-maximal time of 15 min at submillimolar concentrations of ceruloplasmin and of NO (18), are slow compared with the more efficient (k = 2.3 × 10⁶ M⁻²·s⁻¹) reaction of NO with molecular oxygen (9).

Also note that a high oxygen tension was present in our experimental conditions (i.e., pH 7.4, high oxygen buffers and that oxygen also continuously flows at the cell surface under our conditions. The possibility that, when exposed to the aorta, ceruloplasmin enters turnover conditions (i.e., its copper atoms are partially reduced) and it traps NO in this state, analogously to the known inhibition by NO turning over cytochrome c oxidase (37), is also unlikely, since we have previously shown that the affinity of the copper sites of ceruloplasmin for NO is not appreciably dependent on the metal reduction state (27).

Other lines of evidence help to rule out the “trapping” hypothesis. As shown in Fig. 1, the effect is dose dependent; however, ceruloplasmin never totally abolishes the effect of the vasodilator. In fact, its effect levels off at ∼75% of inhibition at infinite concentration. This suggests that ceruloplasmin is not competing with other targets for NO. As a matter of fact, hemoglobin, which readily binds NO in the presence of oxygen, completely blunts the acetylcholine-induced relaxation (20). S-nitrosation of the free cysteine of ceruloplasmin, analogously to that reported for albumin (36), can be ruled out, since ceruloplasmin did not show to be prone to this modification either with authentic NO in the presence of oxygen or by transnitrosation with GS-NO or BSA-NO. This result is probably because of 1) the reduced accessibility of the thiol, as confirmed by the 20-fold difference in reactivity of DTNB toward ceruloplasmin vs. BSA; and 2) the fact that the high reactivity of the cysteine of albumin is mostly due to an abnormally low pK value (36), which is probably not the case for ceruloplasmin. Also note that formation of nitrosothiols like BSA-NO has a stabilizing effect on NO, improving rather than inhibiting its vasorelaxing properties.

It has been shown that iron-dinitrosyl complexes exist bound to the vascular endothelial wall and are capable of reaction with luminal components (24). Because it has been suggested that these complexes may represent the physiological form of EDRF (42), the possibility exists that ceruloplasmin prevents relaxation of the aorta by interacting with them. However, our EPR experiments did not reveal any significant interaction between ceruloplasmin and DNIC 1:20, and the rate of NO release by DNIC 1:20 was essentially unchanged by ceruloplasmin. Furthermore, a high-molecular-weight iron-dinitrosyl like BSA-DNIC was not destabilized by ceruloplasmin, suggesting that also in this respect ceruloplasmin is not simply a scavenger.

Ceruloplasmin could act through its ferroxidase activity by enhancing redox reactions between contaminant iron and NO. For instance, the iron-mediated conversion of NO to NO¹ would be enhanced in the presence of a system capable of recycling Fe(II) to Fe(III). This hypothesis, however, has to be discarded, since under our experimental conditions (i.e., pH 7.4, high oxygen tension and micromolar concentrations of ceruloplasmin) the enzymatic conversion of Fe(II) to Fe(III) is expected to be only slightly more efficient than the spontaneous oxidation of divalent iron (7). If contaminant iron plays a role by itself, heat-treated ceruloplasmin should be as active as the native protein.

A second mechanism would involve a direct interaction of the protein with the vasodilator and/or with the agonist receptor on the endothelial membrane. Although this hypothesis cannot in principle be ruled out, it appears unlikely from the fact that ceruloplasmin equally inhibits the endothelium-dependent relaxation induced by two very different agonists, acetylcholine and ADP. A careful analysis of the data shown in Fig. 1 helps to rule out this second hypothesis. Should ceruloplasmin act through binding and sequestering acetylcholine, the rise in the relaxation value, which is observed at higher concentrations of the agonist and which is known to be due to the direct vasoconstrictor action of acetylcholine on smooth muscle cells (6), should not be observed at the same concentration of the agonist in the presence or absence of ceruloplasmin, at variance with the experimental data. Moreover, calculations performed on the same data presented in Fig. 1
reveal that, whatever the maximal relaxation value obtained in the various conditions, the concentration of acetylcholine needed to achieve half of that maximal relaxation is essentially the same (independent of the presence of ceruloplasmin), suggesting that the protein is not competitively impairing the interaction of the agonist with its receptor.

It is difficult at this stage to envisage the exact mechanism of the action of ceruloplasmin, since our data only safely exclude the trapping hypothesis. The alternative physiological roles of ceruloplasmin (i.e., as a ferroxidase and as a copper transport protein) both seem apparently unrelated to the impaired vasorelaxation observed in the presence of the protein, although others (4, 12, 30) studying the copper transport role of ceruloplasmin have demonstrated that the protein interacts with a number of cellular types and tissues, promoting copper entry possibly after binding to membrane receptors. It remains to be established whether these functionalities can be related to our observations.

Whatever the mechanism governing the inhibition of the endothelium-dependent action of vasodilators by ceruloplasmin, it remains that the phenomenon might have an extraordinary physiological relevance. Ceruloplasmin exerts its effect at micromolar concentrations, well within the range normally found in the plasma. Moreover, as an acute phase protein, the concentration of ceruloplasmin increases severalfold during a number of pathological conditions (16), easily reaching the levels at which the maximum effect on the aorta was observed. It might be speculated that these changes are accompanied by concomitant alterations in the effects of endogenous EDRF. In this respect, it is interesting to note that epidemiological observations have shown an association between increased serum ceruloplasmin (31) or serum copper (32) concentration (which is an indirect measure of ceruloplasmin levels) and the occurrence of acute vascular events such as myocardial infarction and stroke. Even more interesting is the fact that impaired endothelium functionality has been linked to a number of pathological processes, including atherosclerosis (18, 41), and that ceruloplasmin levels in the blood are positively associated with the severity of coronary atherosclerosis (19, 22). It is unfortunate that, to date, no data are available on the vascular status of aceruloplasminemic individuals, who completely lack ceruloplasmin in their blood.

Taken together, the data of the present study suggest that ceruloplasmin could be involved in the physiological control of vascular tone and shed some light on a possible physiological role of this puzzling protein. Work is in progress to elucidate the mechanism of action of ceruloplasmin at the cellular level.

This work was supported in part by Ministero dell’ Università e della Ricerca Scientifica e Tecnologica 40% Funds and by Consiglio Nazionale delle Ricerche Grant 9502167.

Address for reprint requests: G. Musci, cb Dept, of Biochemical Sciences, Univ. La Sapienza, piazzale Aldo Moro, 500185 Rome, Italy. Received 7 July 1997; accepted in final form 19 September 1997.

REFERENCES


