Inhibition of Endothelial Nitric-oxide Synthase by Ceruloplasmin*

(Received for publication, January 7, 1999, and in revised form, April 21, 1999)

Andrea Bianchini‡, Giovanni Musci§, and Lilia Calabrese¶**

From the ‡Department of Biochemical Sciences, University La Sapienza, Piazzale Aldo Moro 5, 00185 Rome, Italy, §Department of Organic and Biological Chemistry, University of Messina, Salita Sperone 31, 98168 S. Agata Messina, Italy, ¶Department of Biology, University Roma Tre, Viale Marconi 446, 00146 Rome, Italy, and ||CNR Center of Molecular Biology, University La Sapienza, Piazzale Aldo Moro 5, 00185 Rome, Italy

The plasma copper protein ceruloplasmin (CP) was found to inhibit endothelial nitric-oxide synthase activation in cultured endothelial cells, in line with previous evidence showing that the endothelium-dependent vasorelaxation of the aorta is impaired by physiological concentrations of ceruloplasmin. The data presented here indicate a direct relationship between the extent of inhibition of agonist-triggered endothelial nitric oxide synthase activation and CP-induced enrichment of the copper content of endothelial cells. Copper discharged by CP was mainly localized in the soluble fraction of cells. The subcellular distribution of the metal seems to be of relevance to the inhibitory effect of CP, because it was mimicked by copper chelates, like copper-histidine, able to selectively enrich the cytosolic fraction of cells, but not by copper salts, which preferentially located the metal to the particulate fraction.

Ceruloplasmin (CP) is a copper-containing glycoprotein that is found in the plasma of all vertebrates, in which it carries approximately 90% of plasma copper (1). Each CP molecule tightly binds six copper atoms to the three different copper binding sites that characterize blue oxidases (2, 3). Nevertheless, as indicated by considerable experimental evidence, it is particularly prone to transfer its copper atoms to tissues (4–6) delivering copper to intracellular copper proteins (7, 8). However, recent studies on aceruloplasminemic patients (9–11) indicate that this protein has no essential role in copper transport, whereas it plays a primary role in iron homeostasis, possibly through its ferroxidase activity (12).

Ceruloplasmin is an acute-phase reactant; its concentration in the plasma increases up to 3-fold during pregnancy and during multiple pathological processes including trauma and inflammation (13). Recent attention has focused on the role that this protein may have in the function of the vascular system in health and disease. CP has been detected in human atherosclerotic lesions (14, 15), and it has been shown to oxidize low density lipoproteins in the presence of vascular cells (16). A copper binding site labile to Chelex treatment has been proposed to be responsible for the oxidative damage to low density lipoproteins (17). On the other hand, we have shown that CP, at physiological concentrations, inhibits the endothelium-dependent relaxation of rabbit aorta induced by agonists and that this effect is not due to a trapping of NO by the copper sites (18).

Vasodilation requires a (NO)-cGMP transduction pathway between endothelium and smooth muscle cells (19, 20). Endothelial NO synthase (eNOS) is a constitutive enzyme that converts L-arginine into NO and citrulline (21) with a relatively low basal activity (22, 23). After agonist stimulation evoking an increase in the [Ca2+]i concentration, Ca2+-bound calmodulin disrupts the inhibitory eNOS-caveolin-1 interactions (24, 25), thereby allowing conformational changes within eNOS, leading to the activated form that produces NO (26, 27). The agonist bradykinin (Bk) has a distinct role among vasodilators because it has also been shown that its receptor physically associates with eNOS (28).

In this context, it should be recalled that a regulatory role for dietary copper in the control of vascular functions has been assessed by numerous studies focused mainly on copper deficiency-induced defects of vessels and on the impairment of NO-mediated vasodilation under copper restriction (reviewed in Ref. 29). On the contrary, an ability of copper ions (Cu2+) to relax vessels seems well established. It has been shown that copper enhances the relaxation of precontracted aortic rings evoked by the calcium ionophore A23187 and sodium nitroprusside (30), and it also elevates intracellular cGMP levels evoked by the calcium ionophore A23187 and sodium nitroprusside (30), and it also elevates intracellular cGMP levels and induces relaxation of pulmonary arterial rings (31). More recently, it has been demonstrated that copper induces the activation of eNOS in cultured endothelial cells (32), an event supposed to occur in vivo in hypercupremic states induced by Cu2+ released by ceruloplasmin. This result is apparently in contrast with our previous findings (18), which are in better accordance with the inhibitory effects exerted by divalent metal ions on eNOS (32, 33) as well as on neuronal nitric oxide synthase (34), another constitutive enzyme, when activity measurements are performed on crude cell extracts or on the purified enzyme (35).

To clarify the mechanism underlying the inhibitory effect of CP on the relaxation of rabbit aortic vessels (18), we tested whether this protein could affect the agonist-induced activation of eNOS in cultured OAECs. Here we show that exogenously added CP reversibly reduces the formation of cGMP, nitrite, and nitric oxide, but not Cu2+ mobilization in endothelial cells stimulated with agonists, with a time course consistent with that of copper delivery to cells, and we show that copper bound to histidine, but not free copper, mimics the effect of CP. Altogether, the results are consistent with a mechanism...
whereby intracellular, CP-derived copper inhibits eNOS activation by agonists.

**EXPERIMENTAL PROCEDURES**

**Materials**—All reagents were purchased from Sigma Italia (Milan, Italy) unless otherwise noted and were used without further purification. Radioactive chemicals were from Amersham Pharmacia Biotech (Milan, Italy).

**Cell Culture**—OAEs were harvested from the internal surface of aorta according to a previously described procedure (36) using collagenase XI and grown in a culture medium containing DMEM (Life Technologies, Inc.) supplemented with 10% FBS (Life Technologies, Inc.). 30 μg/ml endothelial cell growth supplement (Sigma Italia), 100 units/ml penicillin, 100 μg/ml streptomycin, and 50 μg/ml gentamicin (Life Technologies, Inc.) at 37 °C under an atmosphere of 5% CO2 in air. Their identity was verified by their morphological features and immunofluorescence staining with antibodies to factor VIII. The confluent monolayers were subcultured by conventional trypsinization. For the present study, cells were used at the third to sixth passages. Stimulation by agonists was carried out with confluent cells in either modified Hanks’ balanced salt solution (m-HBSS), serum-free DMEM, or DMEM supplemented with 10% FBS (DMEM/FBS). To remove possible traces of thrombin, all CP samples were treated with benzenamide-Sepharose (Amersham Pharmacia Biotech, Uppsala, Sweden) immediately before addition to cells.

**Preparation of Ceruloplasmin**—Sheep and human CP were purified as described previously (2, 37) and further purified by mono Q fast protein liquid chromatography to remove traces of prothrombin. The resulting proteins were >95% pure as judged by spectroscopic and electrophoretic analyses. In some experiments, purified CP samples were treated with Chelex 100 (38). Apoceruloplasmin (ApoCP) was prepared as described previously (39). ApoCP had a residual oxidase activity of ~10% with respect to the native protein, consistent with the presence of ~10% unrecovered copper. Neutralization of CP with anti-CP antibody was carried out by preincubating CP on ice for 30 min with a 2-fold excess of a polyclonal specific anti-sheep ceruloplasmin antibody (kindly provided by Dr. Marmocchi) before the treatment of cells.

**cGMP Assay**—To determine [cGMP], levels in cultured OAEs, cells were preincubated in complete DMEM for 30 min at 37 °C under 5% CO2 with 1 mM isobutylmethylxanthine, a phosphodiesterase inhibitor. The mixture was loaded on a 1-ml column of Dowex 50WX8 pre-equilibrated in 50 mM Tris/HCl, pH 7.4, containing 120 mM NaCl, 15 mM KCl, 5 mM MgCl2, 1 mM EDTA, 1 mM dithiothreitol, 1 mM phenylmethylsulfonlfyl fluoride, 10 μg/liter leupeptin, 10 μg/liter pepstatin, 10 μg/liter aprotinin, and 20 mM CHAPS. The suspension was homogenized in ice with a mini-potter apparatus and then centrifuged at 10,000 × g for 10 min at 4 °C. 20–50-μl aliquots of the supernatants were assayed for cGMP activity by the addition of 50 mM Tris/HCl, pH 7.4, containing 1 mM NADPH, 1.25 mM CaCl2, 1 mM dithiothreitol, 1 mM ADP, 15 mM MgCl2, 1 mM EDTA, 15 mM 6-terohydrobiotinin, 1 mM FAD, 1 mM FMN, 0.1 mM calmodulin, and 15 μCi/ml [3H]arginine and [3H]arginine (total volume, 150 μl). After incubation at 37 °C for 60 min, the reaction was stopped with 5 volumes of 20 mM Heps, pH 5.5, containing 10 mM EDTA. The mixture was loaded on a 1-ml column of Dowex 50WX5 pre-equilibriated in the latter buffer. The eluate was counted for radioactivity to evaluate the extent of [H]citrulline formation. The effect of copper was assessed by adding increasing concentrations of CuCl2 to the homogenate 5 min before adding the cofactors.

**Western Blotting**—Cell lysates were separated by SDS-polyacrylamide gel electrophoresis. The proteins were electrophoretically transferred onto polyvinylidene difluoride membranes, immunodetected with either anti-eNOS (1:1000, Transduction Laboratories) or anti-CP and visualized by enhanced chemiluminescence (Amersham Pharmacia Biotech).

**Copper Transport Experiments**—Cells were incubated with the indicated amounts of CP or copper-histidine, prepared by adding 1 mol of CuCl2 to 3 mol of histidine in water, or CuCl2, in DMEM/FBS, DMEM or m-HBSS. At the end of the incubation, cells were washed three times with PBS containing 1 mM EDTA and either lysed in 10 mM Tris/HCl buffer, pH 7.4, or collected by scraping and homogenized by 30 strokes in a mini-Potter apparatus in ice-cold homogenization buffer (50 mM Tris/HCl buffer, pH 7.4, containing 120 mM NaCl, 15 mM KCl, 5 mM MgCl2, 1 mM EDTA, 10 μg/liter leupeptin, 10 μg/liter pepstatin A, 10 μg/liter aprotinin, and 1 mM phenylmethylsulfonlfyl fluoride) and centrifuged for 60 min at 10,000 × g. The pellets were resuspended in 50 mM Tris/HCl buffer, pH 7.4, containing 1% Triton X-100. Copper content was determined on aliquots of lysates, homogenates, supernatants, and pellets after digestion with HNO3 by flameless atomic absorption (Perkin Elmer 3030 spectrometer equipped with graphite furnace). The data were normalized by dividing picomoles Cu by mg proteins. Proteins were determined using either the biuret method (43) or the bichochomic acid reagent (Pierce).

**Calcium Mobilization Assay**—Cells were grown at confluence in chamber slides (Lab-Tek; Nunc, Naperville, IL) and treated with 4 μM fura 2-acetoxymethyl ester for 45 min at 37 °C to monitor variations of cytosolic free Ca2+ concentrations (44). Emission fluorescence at 510 nm was measured upon excitation at 340 nm and 380 nm and expressed as F340/F380.

**RESULTS AND DISCUSSION**

**Effects of CP on eNOS Activation**—Nitric-oxide synthase is activated in endothelial cell cultures by several agonists including bradykinin and acetylcholine. Because this leads to NO-dependent activation of endothelial guanylate cyclase (45), the rise of the intracellular cyclic GMP concentration, [eGMP], was used as an index of eNOS activity. Untreated OAEs have a basal level of eGMP that was not affected by CP (Fig. 1A). Bk induced an approximately 6-fold increase of [eGMP], that was abolished by inhibition of eNOS by 1 mM l-NAME. When added to cells 15 min before stimulation, CP had a strong, dose-dependent inhibitory effect on the agonist-induced increase of eGMP levels, with a maximal effect at 10 μM concentration. The inhibition was already evident at 1 μM CP and required the native form,
because copper-free CP had a small effect, the magnitude of which was accounted for by the presence of approximately 10% residual active holoprotein (see "Experimental Procedures"). Treatment of CP samples with Chelex 100 (38) to remove loosely bound copper before the addition to cells had no effects on the results shown. Neutralization by a specific antibody substantially relieved the effect of 10 mM CP. By itself, the antibody affected neither the basal nor the bradykinin-stimulated levels of cGMP.

To examine whether CP altered [cGMP]_i through NO-independent routes rather than affecting eNOS catalytic activity, the eNOS activity was monitored using the conversion of [3H]arginine to [3H]citrulline as a measure of NO synthase activity (Fig. 1B). Stimulation of untreated cells by Bk produced a 3-fold enhancement of citrulline production, which was abolished by l-NAME. Preincubation of cells with CP before stimulation substantially reduced Bk-stimulated citrulline production in the same manner as that observed for cGMP production, indicating that the lower levels of [cGMP], attained by cells stimulated in the presence of extracellular CP were indeed related to an inhibition of eNOS activity. Consistent with this finding, CP strongly hindered the release of NO in the medium.

Human CP was also effective in inhibiting the agonist-induced eNOS activation, being ~80% as active as the ovine enzyme. The effect of CP was independent of the signaling pathway responsible for eNOS activation. As revealed by [cGMP], (Fig. 1C) and [3H]citrulline (Fig. 1D), CP exerted a strong inhibitory effect on the response of OAECs to acetylcholine or ADP and on the non-receptor-dependent response to the Ca^{2+} ionophore A23187. Altogether, the data reported in Fig. 1, A-D, show that
the effect of CP is independent of the agonist used to stimulate cells and of the product of eNOS activation that is monitored. The fact that similar end points are obtained with all tested agonists suggests that the effect of CP is exerted on the common target of all agonists, i.e. on eNOS itself.

In agreement with the observation that aortic rings recover the capability to relax after the removal of CP (18), these cells were again found to be responsive to agonists upon the removal of CP and subsequent incubation in fresh medium. However, the results reported in Fig. 1E show that cells that had been exposed to 10 μM CP need 30 min to recover full response to the agonist, either Bk or acetylcholine.

The extent of inhibition of the agonist-induced activation of eNOS depended on the time of exposure of cells to CP. In Fig. 2, the levels of [cGMP], intracellular [3H]citrulline, and nitrite in the medium are shown for cells stimulated with bradykinin at different times after the addition of 10 μM CP to the incubation medium. It should be noted that the indicated times actually encompassed the 10-min time period required for stimulation. It is evident that: (i) the first 15-min preincubation with CP caused an essentially complete (nearly 80%) inhibition of eNOS activity that remained at this level for more than ~60 min, and (ii) a small inhibition occurred when CP was added simultaneously to bradykinin, i.e. zero time of preincubation. Moreover, no significant inhibition was found when CP was added 2 min after the agonist. This experiment was critical because it showed that CP had to be in contact with cells before the agonist in order to exert its effect and was nearly ineffective in suppressing the response of cells once a response had been evoked. It should be noted that the kinetics of formation of citrulline and nitrite (i.e. NO) are similar; however, they differ at short times from that of cGMP. The lag phase observed in the latter case could be explained when we consider that the formation of cGMP requires the secondary activation of guanylate cyclase.

**FIG. 2.** Time course of the inhibitory effect of CP on Bk-induced production of cGMP (■), citrulline (●), and nitrite (▲). Duplicate sets of cells were treated in parallel with 10 μM CP for the indicated times prior to a 10-min stimulation with Bk, and levels of cGMP, [3H]citrulline, and nitrites were assessed. Data are presented as a percentage of the Bk-stimulated control. Data are expressed as the means ± S.D. of at least three independent experiments performed in duplicate. Incubations were performed in DMEM/PBS.

**FIG. 3.** Effect of CP on calcium mobilization in OAECs by bradykinin. 1 μM Bk was added (arrow) to cells loaded with fura 2-acetoxyethyl ester. Cells were untreated (solid line) or treated (dashed line) with 10 μM CP for 15 min before stimulation. The effects shown are representative of five independent experiments. The scale bar (0.2) is shown on the right.
Because agonists activate eNOS through enhancing the free cytosolic calcium concentration, the next question was whether CP could interfere with calcium fluxes in endothelial cells. As indicated by a representative experiment (Fig. 3), the $F_{440}/F_{510}$ fluorescence intensity ratio, which increases upon elevation of $[Ca^{2+}]_i$, varied after stimulation of OAECs by bradykinin, Independently of prior exposure of cells to CP. Similar results were also obtained with A23187, with the ionophore inducing only a bigger variation of the fluorescence intensity ratio (data not shown).

Copper Delivery by CP to Endothelial Cells—It has been shown repeatedly that CP interacts directly with the membrane surface of cells of many tissues, including heart and aorta (46), and that such interactions lead to a transmembrane transport of copper but not of the protein moiety (47). An enhanced metal exportation generally follows the increase of the intracellular concentration of copper in normal cells (48, 49).

To assess the possible effect on eNOS of a transient increase in copper levels, the delivery of copper to OAECs by CP was studied. CP affected the copper content of OAECs in a time-dependent manner (Fig. 4). OAECs exposed to 10 μM CP accumulated copper for at least 60 min, with an almost 7-fold increase, after 60 min, with respect to that of untreated cells (Fig. 4A). Copper uptake by a number of cell types has been the object of intensive investigation, which has also shown that the metal does not easily redistribute among cellular components when cells are homogenized (50). Therefore, analyses of total membranes and cytosolic fractions of treated OAECs were performed and demonstrated that copper was mostly accumulated in the cytosol (Fig. 4B). In contrast, the amount of CP taken by cells or bound to cell membranes, as detected by immunoblot analyses of cell lysates, was at any time approximately 2 orders of magnitude less than the amount of copper (results not shown). As expected, it was also found that the higher the amount of copper extracted by the cells, the lower the amount of copper formed upon stimulation, with an apparently linear relationship (Fig. 4C).

When the copper content was measured on lysates of the cells exposed to CP for 30 min and then washed and left to equilibrate with the medium, the copper level was found to decrease within 30 min to the original level of the untreated cells upon removal of CP (Fig. 4D). These results are consistent with the transient increase in intracellular copper induced by CP as the origin of the inhibitory effect and with the copper efflux mechanism at the basis of cell recovery after CP removal.

Effect of Copper Complexes—If an increase in intracellular copper is required to suppress eNOS activation by agonists, then any copper-donating complex should have the inhibitory effect. Cultured cells are able to take up copper from whatever source is offered, including copper salts and copper-amino acid chelates, although with different transport properties, i.e. kinetics, preference, and path of entry (51).

To this purpose, copper bound to histidine, a complex of physiological relevance for copper transport (50, 52), and copper chloride were tested for their ability to donate copper to OAECs and to impair the bradykinin-induced activation of eNOS. The metal concentrations of the copper salt and of the copper bound to histidine were adjusted to bring the amount of copper to the same level of 1–10 μM CP. Incubations were performed in serum-free media, either DMEM or m-HBSS, to avoid possible interference by serum on the copper status. Copper-histidine was assayed in DMEM to maintain a large excess of histidine, whereas the copper salt was assayed in m-HBSS both to avoid the formation of amino acid chelates and to prevent its calcium-dependent activating effects on eNOS (32).

Control experiments demonstrated that all effects of CP were reproduced under these conditions. As shown in Fig. 5A, the response of cells to the agonist varied depending on the medium; nevertheless, CP was able to exert its effect regardless of the composition of the medium. When cells were incubated for 30 min with copper chloride or copper-histidine (50 μM), there was no overall difference in the uptake of copper with respect to CP (data not shown). Copper bound to histidine reduced the Bk-stimulated production of cGMP in a dose-dependent manner to nearly the same extent as CP (Fig. 5A). On the contrary, CuCl₂ did not exert an inhibitory effect on the agonist-induced response or have any activating effect without the agonist, as expected from the absence of extracellular calcium. The same behavior was observed when the production of [3H]citrulline was monitored (data not shown).

Although copper chloride and the copper-histidine complex appeared to be as efficient as CP in promoting copper enrich-
Ceruloplasmin and eNOS

Ceruloplasmin, the major copper transport protein, plays a crucial role in regulating copper distribution throughout the body. It is involved in the transport of copper from the liver to peripheral tissues, including the liver itself, and is also a key player in the regulation of the production of nitric oxide (NO) by endothelial nitric oxide synthase (eNOS). The interaction between ceruloplasmin and eNOS is complex and involves several mechanisms that are not fully understood.

Recent studies have shown that Cu^{2+} bound to ceruloplasmin (CP) can modulate eNOS activity. The binding of copper to ceruloplasmin can inhibit the activation of eNOS, possibly by altering the conformation of the enzyme or by changing the localization of eNOS within the endothelial cells. This inhibition can be mediated by the formation of a complex between ceruloplasmin and eNOS, which can be affected by the concentration of copper and the availability of other metals.

The precise mechanism by which ceruloplasmin regulates eNOS activity is still under investigation. It is clear, however, that the interaction between these two proteins is important for the normal functioning of the endothelial cells and for the regulation of vascular tone. Further studies are needed to fully understand the role of ceruloplasmin in the control of eNOS activation and its implications for health and disease.

Acknowledgment—We thank the Veterinary Service of ASL Roma B for kindly providing fresh ovine aortas.

REFERENCES

1. Ryden, L. (1984) in Copper Proteins and Copper Enzymes (Lontie, R., ed) Vol. 3, pp. 37–109, CRC Press Inc., Boca Raton, FL.
2. Musci, G., Bonacorsori di Patti, M. C., Fagiolini, U., and Calabrese, L. (1993) J. Biol. Chem. 268, 13398–13392.
3. Zaitseva, I., Zaitsev, V., Card, G., Moskov, K., Bax, B., Ralph, A., and Lindley, P. (1996) J. Biol. Inorg. Chem. 1, 15–23.
4. Marceau, N., and Aspin, N. (1973) Biochim. Biophys. Acta 328, 338–350.
5. Campbell, C. H., Brown, R., and Linder, M. C. (1981) Biochim. Biophys. Acta 678, 27–38.
6. Harris, E. D. (1995) Prog. Clin. Biol. Res. 380, 163–179.
7. Dameron, C. T., and Harris, E. D. (1987) Biochem. J. 248, 669–675.
8. Hsieh, H. S., and Frieden, E. (1975) Biochem. Biophys. Res. Commun. 67, 1326–1331.
9. Harris, Z. L., Takahashi, Y., Serizawa, M., Magillivray, R. T. A., and Gillin, J. D. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 2539–2543.
10. Murita, H., Ikeda, S., Yamamoto, K., Morita, S., Yoshida, K., Nomoto, S., Kato, M., and Yanagisawa, N. (1995) Annu. Rev. Biochem. 64, 646–656.
11. Yoshida, K., Furutaka, K., Takeda, S., Nakamura, A., Yamamoto, K. M., Morita, H., Hiyamuta, S., Ikeda, S., Shimizu, N., and Yanagisawa, N. (1995) J. Clin. Invest. 9, 267–272.
12. Frieden, E., and Heisz, H. S. (1976) Adv. Enzymol. 44, 187–236.
13. Cousins, R. J. (1985) Physiol. Rev. 63, 238–369.
14. Hollander, W., Colombo, M. A., Kirkpatrick, B., and Paddock, J. (1979) Ath-loscleros 44, 391–496.
15. Simms, J., and Gutteridge, J. M. C. (1995) Free Radic. Biol. Med. 20, 1561–1565.
16. Mukhopadhyay, C. K., and Fox, P. L. (1998) Biochemistry 37, 14222–14229.
17. Mukhopadhyay, C. K., Mazumder, B., Lindley, P. F., and Fox, P. L. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 11546–11551.
18. Cappelli-Bigazzi, M., Ambrosio, G., Musci, G., Battagliola, C., Bonacorsori di Patti, M. C., Golino, P., Ragni, M., Chiariello, M., and Calabrese, L. (1997) Am. J. Physiol. 373, H2843–H2854.
19. Schuschnieke, D. A. (1997) J. Nutr. 127, 2274–2281.
20. Plane, P., Wigmore, S., Angelini, G. D., and Jeremy, J. Y. (1997) Br. J. Pharmacol. 121, 345–350.
21. Ohnishi, T., Ishizaki, T., Sasaki, F., Ameshima, S., Nakai, T., Miyamoto, S., and Matsuoka, S. (1997) Eur. J. Pharmacol. 319, 49–55.
22. Demura, Y., Ishizaki, T., Okamura, S., Miyamoto, S., and Matsuoka, S. (1997) Free Radical Biol. Med. 25, 314–320.
23. Howard, A. B., Alexander, R. W., and Taylor, W. R. (1995) Am. J. Physiol. 269, 712–713.
24. Quinlan, M. R., and Harris, C. L. (1995) Neurosci. Lett. 196, 65–68.
25. Persechini, A., McMillan, K., and Siler Master, B. S. (1995) Biochemistry 34, 15091–15099.
26. Gospodarowicz, D., Moran, J., Brown, D., and Birdwell, C. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 4149–4144.
27. Calabrese, L., Carbanaro, M., and Musci, G. (1989) J. Biol. Chem. 264, 6183–6187.
28. Ehrenwald, E., Chisolm, G. M., and Fox, P. L. (1994) J. Clin. Invest. 93, 1453–1501.
29. Matsuoka, S., and Matsuoka, S. (1995) Free Radical Biol. Med. 25, 314–320.
30. Howard, A. B., Alexander, R. W., and Taylor, W. R. (1995) Am. J. Physiol. 269, C612–C618.
31. Quinlan, M. R., and Harris, C. L. (1995) Neurosci. Lett. 196, 65–68.
32. Persechini, A., McMillan, K., and Siler Master, B. S. (1995) Biochemistry 34, 15091–15099.
33. Gospodarowicz, D., Moran, J., Brown, D., and Birdwell, C. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 4149–4144.
34. Calabrese, L., Carbanaro, M., and Musci, G. (1989) J. Biol. Chem. 264, 6183–6187.
35. Matsuoka, S., and Matsuoka, S. (1995) Free Radical Biol. Med. 25, 314–320.
36. Howard, A. B., Alexander, R. W., and Taylor, W. R. (1995) Am. J. Physiol. 269, C612–C618.
37. Quinlan, M. R., and Harris, C. L. (1995) Neurosci. Lett. 196, 65–68.
38. Persechini, A., McMillan, K., and Siler Master, B. S. (1995) Biochemistry 34, 15091–15099.
39. Gospodarowicz, D., Moran, J., Brown, D., and Birdwell, C. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 4149–4144.
40. Calabrese, L., Carbanaro, M., and Musci, G. (1989) J. Biol. Chem. 264, 6183–6187.
41. Ehrenwald, E., Chisolm, G. M., and Fox, P. L. (1994) J. Clin. Invest. 93, 1453–1501.
42. Matsuoka, S., and Matsuoka, S. (1995) Free Radical Biol. Med. 25, 314–320.
43. Howard, A. B., Alexander, R. W., and Taylor, W. R. (1995) Am. J. Physiol. 269, C612–C618.
44. Quinlan, M. R., and Harris, C. L. (1995) Neurosci. Lett. 196, 65–68.
45. Persechini, A., McMillan, K., and Siler Master, B. S. (1995) Biochemistry 34, 15091–15099.