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The effect of Cu^{2+} on rat pulmonary arterial rings

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Abstract

In the current study, Cu^{2+} was tested for its ability to relax vessels and to accumulate cyclic GMP (cGMP) in rat pulmonary artery employing rat extrapulmonary arterial rings. Cu^{2+}-induced relaxation was endothelium and concentration (in the range from 10^{-7} to 10^{-4} M) dependent. The content of cGMP in the rings was increased 1.7-fold with 10^{-4} M Cu^{2+}. N^G-Monomethyl-L-arginine abolished both the copper-induced relaxation and the increase in cGMP of rings. Cu^{2+} and zaprinast, which inhibits phosphodiesterase activity, caused a synergistic increase in cGMP level in the rings, suggesting that Cu^{2+} enhanced cGMP level through a mechanism different from that of zaprinast, probably as a consequence of elevated accumulation of nitric oxide (NO). The magnitude of vasorelaxation observed due to simultaneous addition of Cu^{2+} and acetylcholine was additive, not synergistic. Cu^{2+} did not augment relaxation induced by exogenously added NO donor. These results suggest that Cu^{2+} elevates NO level in the rings not by prolonging the half-life of NO, but by activation of endothelial nitric oxide synthase and subsequently potentiating the action of NO on vascular tone.

Keywords: Cu^{2+}; Nitric oxide (NO); cGMP; Nitric oxide (NO) donor

1. Introduction

Dietary Cu^{2+} deficiency in experimental animals produces vascular defects which include structural changes in aortas (Hunsaker et al., 1984), alteration of vasoactive responses to norepinephrine in portal veins and pulmonary arteries (Kitano, 1980), enhancement of microvascular protein leakage in response to endogenous histamine release (Schuschke et al., 1989), and reduction of microcirculatory platelet thrombus formation in response to photoactivation of a light-sensitive dye. Saari (1992) reported that dietary copper deficiency reduced the relaxation responses to acetylcholine, histamine, or sodium nitroprusside in rat aortic rings probably by disruption of the interaction of endothelium-derived relaxing factor (EDRF) with smooth muscle. In the present study, we examined the effects of Cu^{2+} on the vascular tone and tissue guanosine 3′,5′-cyclic monophosphate (cGMP) level in rat pulmonary arterial rings. We further assessed the effect of Cu^{2+} on the vascular tone during exogenous nitric oxide (NO) donor treatment.

2. Materials and methods

2.1. Preparation of rat extrapulmonary arterial rings and tension recording

Male Sprague-Dawley rats weighing 280–360 g (Clea Japan) were kept in quarantine in our animal care facility for 7 days prior to starting the experiment. Isolation of pulmonary artery was done by the method described previously (Ishizaki et al., 1995). Briefly, animals were anesthetized with pentobarbital (50 mg/kg) intraperitoneally. Then the chest was opened, and heparin sulfate (100 IU) was injected into the right ventricle. The heart and lungs were removed en bloc and placed in Earle's balanced salt solution (EBSS) buffer at 4°C. By using a dissecting microscope and small iris scissors, adventitial tissue was removed and pulmonary artery rings cut. The external diameter of the pulmonary artery used was 2–3 mm and its length 2–4 mm. Care was taken to avoid inadvertent damage to the endothelium. Endothelium-denuded rings were prepared by gently abrading the intima with a roughened steel rod. Rings were mounted on steel wires in organ baths containing 10 ml EBSS buffer at 37°C and gassed

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with 21% O_2, 5% CO_2 and 74% N_2. EBSS had the following composition (in mM): 1.80 $CaCl_2$, 0.83 $MgSO_4$, 5.36 KCl, 116.34 NaCl, 0.40 $NaPO_4$ (dibasic), 5.50 glucose, 19.04 $NaHCO_3$ and 0.03 phenol red Na (as a pH indicator). Tension was measured isometrically using force-displacement transducers (Orientic T7-30-240) and recorded on 4-channel polygraphs (Nihon Kohden RMP-6004M, Japan). Resting passive tension was maintained at 500 mg throughout the experiment. After 60 min of equilibration, all rings were contracted briefly with 80 mM potassium chloride (KCl) as a measure of maximal smooth muscle contractility, and readjusted to the 500 mg resting tension. The rings were then reconstricted with 10^{-6} M phenylephrine and after reaching a maximal constriction, 10^{-6} M acetylcholine was added as a measure of integrity of the endothelium. The responses to acetylcholine of endothelium-intact rings used in these experiments were $79.5 \pm 5.7\%$ ($n = 160$), and those of denuded rings were $5.5 \pm 1.3\%$ ($n = 6$). After the rings were flushed with fresh EBSS buffer three times, they were reequilibrated to the 500 mg resting tension.

2.2. Effect of divalent metal ions on isometric tension

In all experiments, 10^{-5} M indomethacin was added 10 min before the second addition of phenylephrine in order to avoid the effects of cyclooxygenase metabolites. After reaching the maximum vasoconstriction to 10^{-6} M phenylephrine, cumulative concentrations (10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} M) of copper sulfate ($CuSO_4$, $n = 6$) or copper chloride ($CuCl_2$, $n = 6$) were added at 5 min intervals (because maximal stable relaxation was obtained within 5 min). In some experiments, N^G-monomethyl-L-arginine (L-NMMA, 10^{-4} M, $n = 6$), a NO synthase inhibitor, or 2-phenyl-4,4,5,5,-tetramethyl-imidazoline-1-oxyl-3-oxide (PTIO, 3×10^{-4} M, $n = 6$), a NO trapper (Akaike et al., 1993) was added to the organ bath at the time of second phenylephrine administration. The effects of manganese chloride ($MnCl_2$, $n = 6$), iron chloride ($FeCl_2$, $n = 6$), zinc chloride ($ZnCl_2$, $n = 6$) and calcium chloride ($CaCl_2$, $n = 6$) on isometric tension of rings preconstricted with 10^{-6} M phenylephrine were additionally assessed in other rings. Isometric tension was recorded and the relaxations were plotted as the percentage of the contraction induced by 10^{-6} M phenylephrine.

2.3. Evaluation of intracellular cGMP content

The pulmonary arterial rings were mounted in the organ baths as described above. In these experiments, zaprinast (type V, cGMP-specific phosphodiesterase inhibitor; 100 μM) was also added 10 min before administration of phenylephrine. The maximal dilation was observed about 2 min after addition of $CuCl_2$. At the point of maximal dilation, the buffer in the organ baths was discarded and the rings were rapidly frozen under liquid nitrogen. Vehicle-control (distilled water) rings were also frozen in the same fashion. The rings were freeze-dried for 24 h, and weighed. Cyclic GMP was extracted by homogenizing the frozen rings in 1 ml of ice-cold 6% trichloroacetic acid and by centrifuging the homogenate at 3000 rpm for 15 min. Extraction was then performed in duplicate with 5 ml diethyl ether. Measurements were made in duplicate on 100 μl aliquots with a radioimmunoassay kit (Yamasa-shoyu, Tokyo, Japan). cGMP content was expressed as picomoles per mg dry weight of pulmonary arterial ring tissue.

2.4. Effect of $CuCl_2$ and acetylcholine on the tension of pulmonary arterial rings

In order to compare the relaxant effect of Cu^{2+} and acetylcholine, different concentrations of $CuCl_2$ (10^{-6} M, 10^{-5} M and 10^{-4} M) and acetylcholine (10^{-7} M, 2×10^{-7} M and 3×10^{-7} M) were added at the same time. Namely different concentrations of $CuCl_2$ and acetylcholine were added alone or simultaneously and percent dilatation was assessed.

2.5. Effect of $CuCl_2$ on relaxation induced by NO donors

To assess the effect of $CuCl_2$ on a NO donor, 3-(2-hydroxy-1-methyl-2-nitrosohydrazino)-*N*-methyl-1-propanamine or NOC 7 (Dojindo, Kumamoto, Japan) induced vasorelaxation at concentrations of 10^{-9} M, 10^{-8} M, 10^{-7} M and 10^{-6} M, these were sequentially added to the phenylephrine-preconstricted rings at 3 min interval (3 min being enough to reach the plateau vasorelaxatory effect of NOC 7) in the presence or absence of $CuCl_2$. 10^{-4} M L-NMMA was added as a pretreatment to inhibit endogenous NO synthase. When NOC 7 was added to the neutral buffer (pH 7.4), it immediately released double molar NO, the half-life ($t_{1/2}$) of which has been reported to be 5 min (Dojindo, unpublished observation).

2.6. Reagents

EBSS, $CuCl_2$, phenylephrine hydrochloride, acetylcholine chloride, and zaprinast were obtained from Sigma (St. Louis, MO, USA). $CuSO_4$, $MnCl_2$, $FeCl_2$, $ZnCl_2$, and $CaCl_2$ were from Wako (Tokyo, Japan). L-NMMA and PTIO were purchased from Calbiochem (La Jolla, CA, USA) and Tokyo Kasei Kogyo (Tokyo, Japan), respectively. Indomethacin was a gift from Yamanouchi Pharmaceutical (Tokyo, Japan). The NO donor, NOC 7, was from Dojindo (Kumamoto, Japan). PTIO and zaprinast were dissolved in 99.9% ethanol (final concentration of ethanol was 0.30%) and 0.1 M NaOH, respectively. NOC 7 was dissolved in 0.1 M NaOH, and further dissolved in 0.02 M NaOH just before using. All other drugs were prepared in distilled water. All concentrations were expressed as final concentration in the organ bath solution.

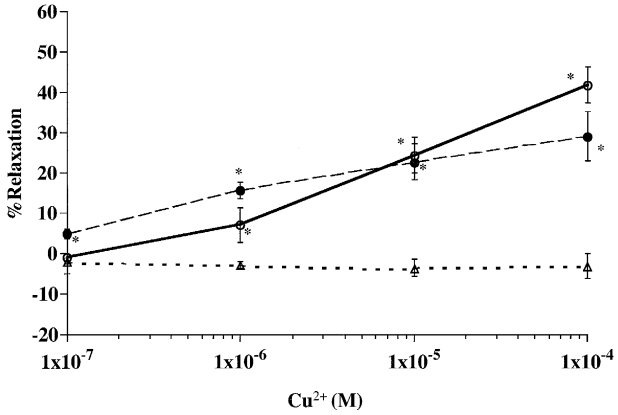

Fig. 1. Dose-dependent vasodilatory effects of Cu^{2+} (as $CuCl_2$ (●) and $CuSO_4$ (○)) on rat endothelium-intact pulmonary arterial rings. Rat pulmonary arterial rings were preconstricted with 10^{-6} M phenylephrine. The data were calculated as a percentage of the maximal constriction. Values are mean ± S.E.M. of six experiments. * $P < 0.05$ (vs. vehicle control (△)).

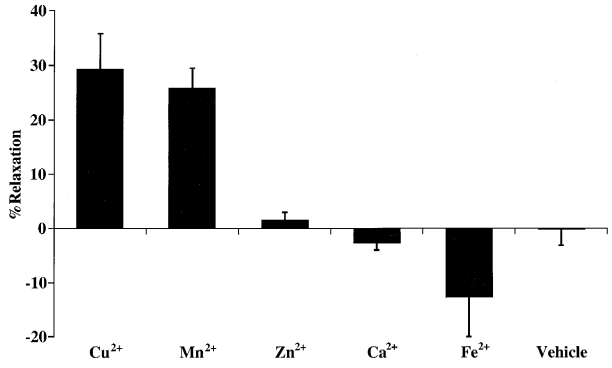

Fig. 2. Effects of various divalent metal ions on rat pulmonary arterial rings. Rat pulmonary arterial rings were preconstricted with 10^{-6} M phenylephrine. Concentration of each ion was 10^{-4} M. Values are mean ± S.E.M. of six experiments.

2.7. Statistics

All values are presented as mean ± S.E.M. Statistical evaluation of each value was performed using a one-way analysis of variance with Bonferroni for multiple comparisons. Results of curves were compared by using two-way factor (treatment and concentration) analysis of variance for repeated measures. If the P value was significant, a one-way analysis of variance with Bonferroni for multiple comparisons was employed to allow comparison of individual means. Values were considered to be statistically significant when the P values were less than 0.05.

3. Results

3.1. Effects of divalent metal ions on isolated pulmonary arterial rings preconstricted with phenylephrine

Both $CuSO_4$ and $CuCl_2$ (10^{-7}–10^{-4} M) dose-dependently dilated phenylephrine-preconstricted pulmonary arterial rings (Fig. 1). A maximal relaxation to $CuCl_2$ (29.3 ± 6.5%) and to $CuSO_4$ (41.9 ± 6.1%) was obtained with 10^{-4} M Cu^{2+} in endothelium-intact rings. Endothelium denudation significantly attenuated the vasodilating effect of $CuCl_2$ (Table 1).

Blunting of the relaxation response in endothelium-intact pulmonary arterial rings was accomplished by pretreatment with L-NMMA (10^{-4} M, Table 1) or PTIO (3×10^{-4} M, Table 1). Among other four divalent metals, $MnCl_2$ dilated pulmonary arterial rings (25.7 ± 3.8% at a dose of 10^{-4} M) preconstricted with phenylephrine as shown in Fig. 2, while $FeCl_2$ inversely augmented the vasoconstriction. Neither $ZnCl_2$ nor $CaCl_2$ altered phenylephrine-induced constriction.

3.2. Detection of cGMP in pulmonary arterial rings

Tissue concentrations of cGMP increased 1.7-fold over the control tissue in response to $CuCl_2$ at a dose of 10^{-4} M (Fig. 3). Increase in tissue cGMP content in response to $CuCl_2$ was inhibited and showed a lower level than the control level by pretreatment with L-NMMA (10^{-4} M). When pulmonary arterial rings were treated with acetylcholine (10^{-6} M), the cGMP levels increased 4-fold over control levels (data not shown). We then examined the effect of zaprinast, type V, a cGMP-specific phosphodiesterase inhibitor, on the baseline and Cu^{2+}-induced cGMP levels. During the preincubation of the pulmonary arterial rings with 100 μM zaprinast at 37°C for 10 min, the resting level of cGMP increased about 1.9-fold in the vehicle control, whereas 10^{-4} M Cu^{2+} elevated the cGMP

Table 1
Vasorelaxant effect of Cu^{2+} on rat pulmonary arterial rings precontracted with 10^{-6} M phenylephrine

Concentration of Cu^{2+} (M)	10^{-7}	10^{-6}	10^{-5}	10^{-4}
Endothelium-intact ring	5.2 ± 1.5	15.9 ± 3.4	22.9 ± 5.2	29.3 ± 6.5
pretreated with 10^{-4} M L-NMMA	−0.6 ± 0.5 [a]	−0.1 ± 0.9 [a]	3.3 ± 1.1 [a]	6.9 ± 2.0 [a]
pretreated with 3×10^{-4} M PTIO	−0.2 ± 0.7 [a]	−0.5 ± 1.0 [a]	0.1 ± 1.5 [a]	1.2 ± 2.1 [a]
Endothelium-denuded ring	−0.9 ± 0.6 [a]	0.9 ± 1.0 [a]	4.0 ± 1.8 [a]	7.7 ± 2.3 [a]

Percent dilatation to various concentrations of Cu^{2+} are shown. L-NMMA and PTIO were added 10 min before administration of phenylphrine. Values are mean ± S.E.M., $n = 6$. [a] $P < 0.05$ vs. endothelium-intact ring.

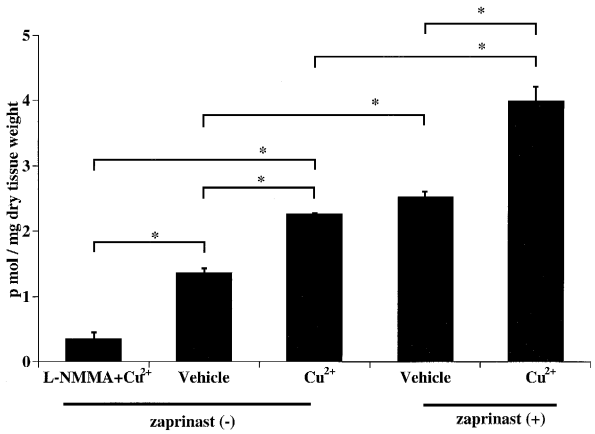

Fig. 3. Amounts of cyclic GMP in rat pulmonary arterial rings. All procedures were performed in the absence or presence of 10^{-4} M zaprinast. Values are mean ± S.E.M. of four experiments. * $P < 0.05$.

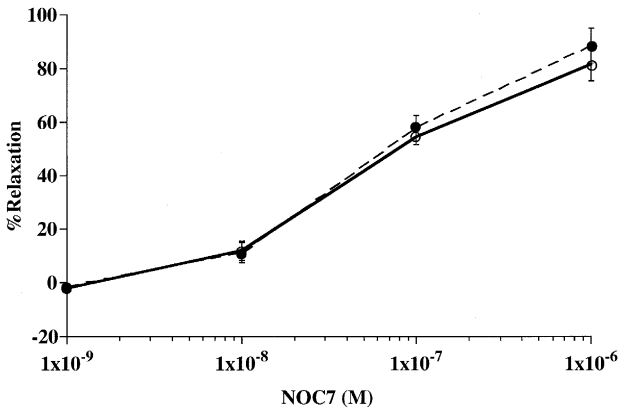

Fig. 5. Effect of $CuCl_2$ pretreatment on relaxation induced by NOC 7. Vasodilatory effects of NOC 7 on rat pulmonary arterial rings pretreated with (○) or without (●) 10^{-5} M $CuCl_2$. Rat pulmonary arterial rings were preconstricted with 10^{-6} M phenylephrine. The data were calculated as a percentage of the maximal constriction. Values are mean ± S.E.M. of four experiments.

content to 1.8-fold compared with 10^{-4} M Cu^{2+} in the absence of zaprinast.

3.3. Effect of $CuCl_2$ and acetylcholine

10^{-7} M acetylcholine and 10^{-6} M $CuCl_2$ dilated pulmonary arterial rings to 13.8 ± 1.5% and 13.5 ± 2.0%, respectively. 26.4 ± 2.3% relaxation was shown when both were added simultaneously (Fig. 4a). 10^{-7} M acetylcholine and 10^{-5} M $CuCl_2$ induced dilatation to 14.2 ± 3.3% and 24.6 ± 4.5%, respectively. 38.5 ± 3.9% relaxation occurred by addition of both (Fig. 4b). 10^{-7} M acetylcholine and 10^{-4} M $CuCl_2$ relaxed the rings by 14.7 ± 1.1% and 35.0 ± 1.0%, respectively. 47.0 ± 1.0% relaxation was noted by addition of both (Fig. 4c). When both acetylcholine (at doses of 2×10^{-7} M and 3×10^{-7} M) and $CuCl_2$ (10^{-6} M, 10^{-5} M) were added simultaneously, additional vasodilatory effects were observed (Fig. 4d–f).

3.4. Effect of $CuCl_2$ on relaxation induced by NOC 7

NOC 7 dose-dependently relaxed rings preconstricted with 10^{-6} M phenylephrine, but 10^{-5} M $CuCl_2$ did not

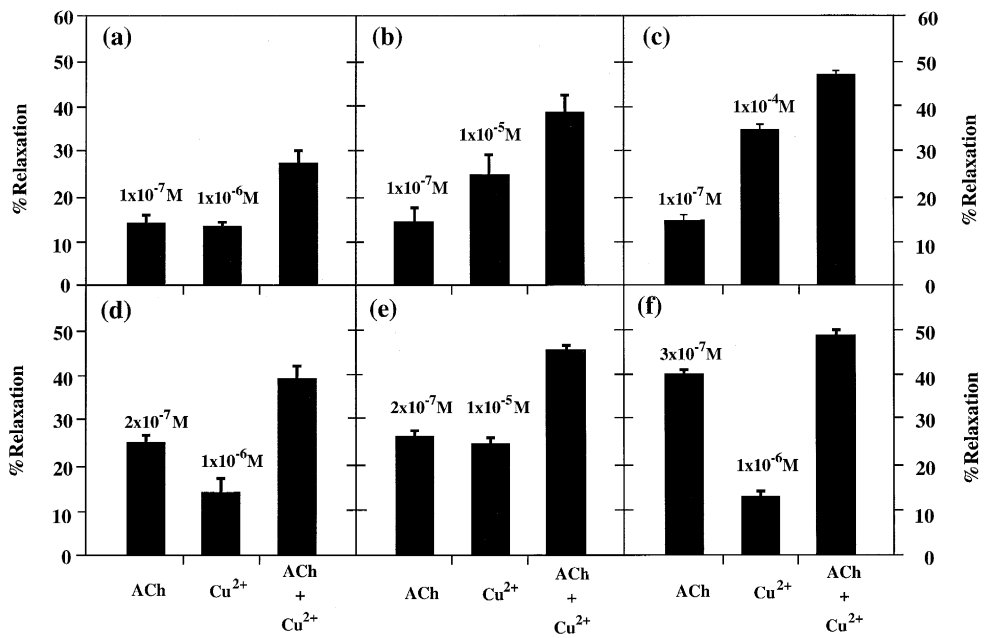

Fig. 4. Effect of acetylcholine and $CuCl_2$ on pulmonary arterial rings. Acetylcholine and $CuCl_2$ were added alone or simultaneously. Values are mean ± S.E.M. of four experiments. 10^{-7} M acetylcholine and 10^{-6} M $CuCl_2$ additionally dilated pulmonary arterial rings to 26.4 ± 2.3% when they were simultaneously added (a). 10^{-7} M acetylcholine and 10^{-5} M $CuCl_2$ (b), 10^{-7} M acetylcholine and 10^{-4} M $CuCl_2$ (c), 2×10^{-7} M acetylcholine and 10^{-6} M $CuCl_2$ (d), 2×10^{-7} M acetylcholine and 10^{-5} M $CuCl_2$ (e), and 3×10^{-7} M acetylcholine and 10^{-6} M $CuCl_2$ (f) showed similar results.

modulate the vasodilatory effect induced by NOC 7 (Fig. 5).

4. Discussion

The major findings in our experiments are that divalent transition metals Cu^{2+} and Mn^{2+} but not Zn^{2+}, Fe^{2+}, or Ca^{2+} caused endothelium-dependent, concentration-dependent vasodilation. Cu^{2+}-dependent vasodilation was inhibited in the presence of either L-NMMA, a nitric oxide synthase inhibitor or PTIO, a NO trapper and Cu^{2+} increased tissue cGMP content of pulmonary arterial rings. Taken together, these results suggest three possibilities: First, Cu^{2+} may activate vascular endothelial NO synthase. Second, Cu^{2+} may prolong the half-life of NO by mechanisms that mimic the action of superoxide dismutase, ultimately resulting in the potentiation of NO activity in vascular tissue. Thirdly, Cu^{2+} stimulates NO release from endogenous *S*-nitrosothiols as Gordge et al. (1995) described. Assuming that the vasodilatory mechanisms of acetylcholine and Cu^{2+} are different, vasorelaxation due to simultaneous addition of both would be synergistic. In this study, the effect of the simultaneous addition of both on their vasorelaxant ability was seen to be additive when compared with that of either acetylcholine or Cu^{2+} alone. This observation further suggested activation of a common vasodilatory pathway by acetylcholine and Cu^{2+}: activation of NO synthase.

What is the mechanism by which Cu^{2+} augments vascular endothelial NO synthase activity? It has been reported that optimal activation of guanylate cyclase via its heme group is required for NO to be in its oxidized (NO^+, nitrosonium) form (Severina et al., 1992). Such an oxidation could occur during the conversion of Cu(II) to Cu(I): redox regulation of soluble guanylate cyclase (White et al., 1976). Palmer and Moncada (1989) further noted that some divalent cations were necessary for the activation of NO synthase. Although we did not assess directly the activity of NO synthase, our results suggest that Cu^{2+} itself would work independently from the element of superoxide dismutase since it has been reported that the action of superoxide dismutase cannot be attributed to the activation of NO synthase; superoxide dismutase failed to increase the formation of L-citrulline from L-arginine (Hobbs et al., 1994), though copper functions in electron transport during the catalytic cycle in Cu,Zn-superoxide dismutase (Fridovich, 1986). Another possible vascular-tone modulation by Cu^{2+} may partly be due to the enhancement of Fenton's type reaction to produce hydroxyl radicals from H_2O_2 (Ookawara et al., 1992), causing changes in the cellular redox state, which may affect NO synthase activity.

As a second possibility, recently Kasten et al. (1994) reported that manganese potentiates NO-mediated vascular relaxation probably by prolonging the half-life of NO by a mechanism mimicking the action of superoxide dismutase, though over 80 μM of manganese lost its vasorelaxant ability. In our study, when a NO donor, NOC 7 (Maragos et al., 1991; Hrabie et al., 1993) was exogenously added in the presence of L-NMMA (in order to inhibit endogenous NO synthase), Cu^{2+} did not potentiate relaxation induced by the NO donor. The experimental result, thereby, negates the possibility of prolongation of the half-life of NO by Cu^{2+}. Thus, although the effects of Cu^{2+} and Mn^{2+} on raising cGMP level in the pulmonary arterial ring system were phenotypically indistinguishable from each other, the effect of Mn^{2+} provides a striking contrast to that of Cu^{2+}, of which the action site is now considered to be activation of NO synthase activity.

Thirdly, Cu^{2+} might stimulate NO release from the endogenous *S*-nitrosothiols since Gordge et al. (1995) described the specific Cu^{2+} chelator bathocuproine sulphonate in washed human-platelet suspension reducing the inhibition of thrombin-induced platelet aggregation by exogenous *S*-nitrosoglutathione and cGMP generation and Cu^{2+} itself enhancing *S*-nitrosoglutathione-induced antiaggregatory activity. We, however, found no additional study to give further insight into this issue.

There are other possible mechanisms of Cu^{2+}-induced relaxation: Does copper directly activate vascular guanylate cyclase? This possibility is, however, negated since the intact endothelium was prerequisite for Cu^{2+}-induced vasorelaxation. The same explanation may be applicable to the argument supporting non-involvement of Cu^{2+} in extending the half-life of cGMP in vascular smooth muscle. The endothelium dependence of vasodilators is generally related to the production and release of two possible types of mediators by the endothelial cell, EDRF or arachidonic acid-derived metabolites, in particular, prostacyclin (Gryglewski et al., 1988). EDRF, which is a NO or a closely related substance (Ignarro et al., 1987; Palmer et al., 1987), is believed to penetrate into the smooth muscle cell, bind and activate guanylate cyclase through binding to its heme moiety, thereby producing cGMP, which in turn causes relaxation by a mechanism not yet delineated. Prostacyclin, on the other hand, causes vasodilation by the activation of adenylate cyclase and resultant production of cyclic AMP (Moncada and Vane, 1979). The latter possibility was negated, because the cyclooxygenase inhibitor, indomethacin, was present throughout the experiments.

In this study we used zaprinast, as a phosphodiesterase V inhibitor (Komas et al., 1991). The effect of the coexistence of zaprinast and Cu^{2+} on the elevation of cGMP level may be accounted for by synergistic activation of guanylate cyclase in response to increased NO which was attained through NO synthase activated by Cu^{2+}, and of suppression of cGMP degradation by phosphodiesterase which was partially inhibited by zaprinast. This hypothetical implication was further verified by the calculation using the experimental data shown in Fig. 3.

Cu^{2+} increases NO production rate without altered degradation rate of NO and raises cGMP level 1.68-fold, that is, from 1.35 to 2.27 (pmol/mg dry weight). However, our present result and a previous report by Kasten et al. (1994) indicated that 100 μM zaprinast suppressed degradation of cGMP through the nonspecific inhibition of phosphodiesterase activity, resulting in a 1.88-fold elevation of intracellular cGMP level, from 1.35 to 2.54 (pmol/mg dry weight). The concurrent treatment of pulmonary arterial rings with Cu^{2+} and zaprinast, therefore, can be expected to be synergistic in increasing intracellular cGMP level because the action site of Cu^{2+} may be different from that of zaprinast. In fact, the experimental data (4.1 pmol/mg dry weight) are in accordance with the predicted cGMP level which was estimated to be 4.26 pmol/mg dry weight on the basis of a synergistic model, namely $1.68 \times 1.88 \times 1.35 = 4.26$. If the effects of Cu^{2+} and zaprinast were simply additive, intracellular cGMP concentration would be estimated to be 3.46 pmol/mg, which would significantly deviate from the present experimental data.

Altura and Altura (1987a,b) reported that magnesium is an important co-factor for acetylcholine-induced endothelium-dependent relaxation in canine coronary arteries, suggesting enhancement of vascular NO synthase activity. On the contrary, Howard et al. (1995) described that magnesium inhibits porcine aortic endothelial cell NO synthase activity, probably due to competitive antagonism of intracellular calcium. In our study, we could not verify any similarity between Cu^{2+} and Mg^{2+} regarding the mechanism of pulmonary vasodilation.

One can raise the question about the pathophysiological relevance of our findings since in human blood vessels all free Cu^{2+} would be bound to ceruloplasmin, a Cu^{2+} carrying plasma protein. However, in the pathological state, Cu^{2+} released from fragmented superoxide dismutase might contribute to vasodilation of local circulation, causing hyperemia. In this regard, Ookawara et al. (1992) reported that site-specific and random fragmentation of superoxide dismutase occurred by glycation reaction and that glycated Cu,Zn-superoxide dismutase is increased in the erythrocytes of patients with diabetes mellitus (Arai et al., 1987).

In conclusion, this investigation demonstrated that in rat pulmonary arterial rings the vasodilating effectiveness of Cu^{2+} was due to activation of NO synthase.

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References

Akaike, T., M. Yoshida, Y. Miyamoto, K. Sato, M. Kohno, K. Sasamoto, K. Miyazaki, S. Ueda and H. Maeda, 1993, Antagonistic action of imidazolineoxyl *N*-oxides against endothelium-derived relaxing factor/·NO through a radical reaction, Biochemistry 32, 827.

Altura, B.T. and B.M. Altura, 1987a, Endothelium-dependent relaxation in coronary arteries requires magnesium ions, Br. J. Pharmacol. 91, 449.

Altura, B.M. and B.T. Altura, 1987b, Peripheral and cerebrovascular actions of ethanol, acetoaldehyde, and acetate: relationship to divalent cations, Alcohol. Clin. Exp. Res. 11, 99.

Arai, K., S. Iizuka, Y. Tada, K. Oikawa and N. Taniguchi, 1987, Increase in the glucosylated form of erythrocyte Cu-Zn-superoxide dismutase in diabetes and close association of the nonenzymatic glucosylation with the enzyme activity, Biochim. Biophys. Acta 924, 292.

Fridovich, I., 1986, Superoxide dismutases, Adv. Enzymol. 58, 61.

Gordge, M.P., D.J. Meyer, J. Hothersall, G.H. Neild, N.N. Payne and A. Noronha-Dutra, 1995, Copper chelation-induced reduction of the biological activity of *S*-nitrosothiols, Br. J. Pharmacol. 114, 1083.

Gryglewski, R.J., R.M. Botting and J.R. Vane, 1988, Mediators produced by the endothelial cell, Hypertension 12, 530.

Hobbs, A.J., J.M. Fukuto and L.J. Ignarro, 1994, Formation of free nitric oxide from L-arginine by nitric oxide synthase: direct enhancement of generation by superoxide dismutase, Proc. Natl. Acad. Sci. USA 91, 10992.

Howard, A.B., R.W. Alexander and W.R. Taylor, 1995, Effects of magnesium on nitric oxide synthase activity in endothelial cells, Am. J. Physiol. 269, C612.

Hrabie, J.A., J.R. Klose, D.A. Wink and L.K. Keefer, 1993, New nitric oxide-releasing zwitterions derived from polyamines, J. Org. Chem. 58, 1472.

Hunsaker, H.A., M. Morita and K.G.D. Allen, 1984, Marginal copper deficiency in rats. Aortal morphology of elastin and cholesterol values in first-generation adult males, Atherosclerosis 51, 1.

Ignarro, L.J., G.M. Buga, K.S. Wood, R.E. Byrns and G. Chaudhuri, 1987, Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide, Proc. Natl. Acad. Sci. USA 84, 9265.

Ishizaki, T., H. Takahashi, T. Ozawa, S.W. Chang and N.F. Voelkel, 1995, Leukotoxin, 9, 10-epoxy-12-octadecenoate causes pulmonary vasodilation in rats, Am. J. Physiol. 268, L123.

Kasten, T.P., S.L. Settle, T.P. Misko, M.G. Currie and G.A. Nickols, 1994, Manganese potentiation of nitric oxide-mediated vascular relaxation, Eur. J. Pharmacol. 253, 35.

Kitano, S., 1980, Membrane and contractile properties of rat vascular tissue in copper-deficient conditions, Circ. Res. 46, 681.

Komas, N., C. Lugnier and J.-C. Stoclet, 1991, Endothelium-dependent and independent relaxation of the rat aorta by cyclic nucleotide phosphodiesterase inhibitors, Br. J. Pharmacol. 104, 495.

Maragos, C.M., D. Morley, D.A. Wink, T.M. Dunams, J.E. Saavedra, A. Hoffman, A.A. Bove, L. Isaac, J.A. Hrabie and L.K. Keefer, 1991, Complexes of ·NO with nucleophiles as agents for the controlled biological release of nitric oxide. Vasorelaxant effects, J. Med. Chem. 34, 3242.

Moncada, S. and J.R. Vane, 1979, Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A_2 and prostacyclin, Pharmacol. Rev. 30, 293.

Ookawara, T., N. Kawamura, Y. Kitagawa and N. Taniguchi, 1992, Site-specific and random fragmentation of Cu,Zn-superoxide dismutase by glycation reaction, J. Biol. Chem. 267, 18505.

Palmer, R.M.J. and S. Moncada, 1989, A novel citrulline-forming enzyme implicated in the formation of nitric oxide by vascular endothelial cells, Biochem. Biophys. Res. Commun. 158, 348.

Palmer, R.M.J., A.G. Ferrige and S. Moncada, 1987, Nitric oxide release

accounts for the biological activity of endothelium-derived relaxing factor, Nature 327, 524.
Saari, J.T., 1992, Dietary copper deficiency and endothelium dependent relaxation of rat aorta, Proc. Soc. Exp. Biol. Med. 200, 19.
Schuschke, D.A., J.T. Saari, D.M. Ackermann and F.N. Miller, 1989, Microvascular responses in copper-deficient rats, Am. J. Physiol. 257, H1607.
Severina, I.S., O.G. Bussygina and N.B. Grigorjev, 1992, Effect of nitroso complexes of some transition metals on the activity of soluble guanylate cyclase, Biochem. Int. 26, 695.
White, A.A., K.M. Crawford, C.S. Patt and P.J. Lad, 1976, Activation of soluble guanylate cyclase from rat lung by incubation or by hydrogen peroxide, J. Biol. Chem. 251, 7304.