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Protection of vascular wall function in insulin-resistant rats from copper oxidative stress

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1 The effects of oxidative stress on vascular function in the insulin-resistant state were assessed in mesenteric resistance arteries of obese, insulin-resistant (cp/cp) and lean, normal (+/?) JCR : LA-cp rats.

2 Nitric oxide-mediated relaxation of noradrenaline-contracted arteries in response to acetylcholine was impaired after 2 h of incubation with Cu^{2+} in both genotypes, with or without the continuing presence of Cu^{2+}. Relaxation was enhanced on initial exposure to Cu^{2+}, and post-incubation removal of the Cu^{2+} resulted in a greater impairment of relaxation. Arteries from cp/cp rats were less impaired in function by Cu^{2+} incubation than were those of +/? controls.

3 Sodium nitroprusside-mediated relaxation was impaired by exposure to Cu^{2+}, with an accompanying increase in EC_{50}.

4 The impairment in acetylcholine-mediated relaxation in the arteries from both cp/cp and +/? rats was completely inhibited by co-incubation with copper-zinc superoxide dismutase and catalase, confirming that the impairment associated with Cu^{2+} incubation was due to oxidative stress.

5 The impairment appears to involve both smooth muscle and the endothelium.

6 The cp/cp rats showed greater resistance to the effects of oxidative stress on arterial function, possibly due to an adaptation to oxidative stress on arterial function associated with the insulin-resistant state.

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Abbreviations: NA, noradrenaline; SNP, sodium nitroprusside; SOD, superoxide dismutase

Introduction

Insulin resistance is a common metabolic abnormality associated with obesity and is present in approximately 25% of the adult population in Western cultures. It is associated with a clustering of cardiovascular risk factors such as hyperinsulinaemia (Després *et al*., 1996), elevated triglyceride levels, low high-density lipoprotein cholesterol, and hypertension (Reaven, 1995). The precise nature of the relationship between insulin resistance and cardiovascular disease is unclear and is complicated by the coexistence of multiple cardiovascular risk factors, but may be associated with hyperinsulinaemia-induced abnormalities in vascular wall function.

Oxidative stress has been proposed to be a causative factor in the development of vascular disease (Salonen *et al*., 1992), and indices of oxidative stress have been reported as being elevated in both humans and rats that are obese and insulin-resistant (Laight *et al*., 1998). Oxidative stress and vascular wall function may interact *via* lipid peroxides in lipoproteins (Auge *et al*., 1996) or possibly by enhancing insulin resistance (Paolisso & Giugliano, 1996). Copper ions (Cu^{2+}) are well known for their ability to initiate lipid peroxidation and have been widely used as initiators of the *in vitro* oxidation of lipoproteins (Frei & Gaziano, 1993). The role of copper *in vivo* is controversial, but it has been identified in atherosclerotic plaques (Iskara *et al*., 1997), and copper-containing plasma proteins such as ceruloplasmin can function as initiators of lipid peroxidation by interacting with superoxide (Mukhopadhyay & Fox, 1998). Copper can also stimulate the proliferation and migration of endothelial cells (Hu, 1998), while a deficiency adversely affects cardiovascular homeostasis (Schuschke, 1997).

Under basal conditions, the smooth muscle of arterial walls has an inherent tendency to constrict the lumen. This basal contractile state is further enhanced by endocrine and neural vasoconstrictor agonists such as noradrenaline (NA), and by vasoconstrictors secreted from the local endothelium. Endothelial cells also play an important role in modifying the contractility of vascular smooth muscle tissue by secreting vasodilatory substances, including nitric oxide (NO) (Moncada *et al*., 1991). The functional response of the vasculature to oxidative stress in the presence of insulin resistance is unknown, but may be an important element in the vasculopathy that is associated with hyperinsulinaemia (Richardson *et al*., 1998).

The JCR : LA-cp rat, when homozygous for the autosomal recessive *cp* gene (cp/cp), lacks membrane-bound leptin

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receptors (ObR), leading to a marked obesity (Wu-Peng *et al*., 1997). The cp/cp animals are hyperphaegic, becoming insulin resistant, hyperinsulinaemic, and hypertriglyceridaemic by 7 weeks of age (Russell *et al*., 1998), and develop advanced atherosclerotic disease as well as myocardial lesions consistent with an ischaemic origin (Russell & Amy, 1986; Richardson *et al*., 1998). Breeding is done using heterozygous animals (cp/+) and yields 25% cp/cp and obese and 75% lean rats (a 2 : 1 mix of cp/+ and +/+ referred to as +/?; for review see O'Brien & Russell, 1997). Hypertension does not develop in this strain, thus providing us with a spontaneous animal model of cardiovascular disease that exhibits all the aspects seen in obese, insulin-resistant humans, including vasculopathy, but without the confounding effects of hypertension.

We compared the functional responses of mesenteric resistance arteries of +/? and cp/cp rats to *ex vivo* oxidative stress in order to assess its role in an animal model of vascular disease. The results show that oxidative stress leads to the impairment of vascular function and that the cp/cp rat is more tolerant of oxidative challenge than is the normal +/? animal.

Methods

Animals

Male cp/cp and +/? rats were raised in our established JCR : LA-cp colony as previously described (Russell *et al*., 1995) and were studied at 6 months of age. The animals were maintained in a controlled environment at 20°C and 40–50% humidity, with 12 h of light per 24 h period. Rat chow (Rodent Diet 5001, PMI Nutrition International, St. Louis, MO, U.S.A.) and tap water were available *ad libitum*. All care and treatment of the rats was in conformity with the guidelines of the Canadian Council on Animal Care and was subject to prior institutional approval as provided for in the guidelines.

All animals were studied in the non-fasted state at the end of the dark phase of their diurnal cycle. The rats were anaesthetized with 3% halothane at 11 min^{-1} O_2. A midline thoractomy was performed, and a section of the mesenteric arcade 5 to 10 cm distal to the pylorus was removed and placed immediately in ice-cold HEPES-buffered physiological saline (in mM: NaCl 142, KCl 4.7, $MgSO_4$ 1.17, KH_2PO_4 1.17, $CaCl_2$ 1.2, HEPES 10, glucose 5). Samples of thoracic aorta were also taken for enzyme assay, immediately frozen in liquid nitrogen, and stored at −70°C until assayed.

Artery preparation

All fat and connective tissue was removed from mesenteric arteries of ~300 μm in diameter. These were then cut to ~2 mm in length and threaded onto two wires of 25 μm diameter attached in an isometric myograph system (Kent Scientific, Litchfield, CT, U.S.A.). The arterial segments rested in 5 ml tissue baths with HEPES-buffered physiological saline at pH 7.4 and maintained at 37°C. Four baths were used per experiment. The force produced was recorded on a data acquisition system (Workbench, Strawberry Tree, Sunnyvale, CA, U.S.A.).

Resting length tension curve

Arteries were stretched to ~0.2 mN mm^{-1} of vessel length (1 mN = 102 mg), allowed to equilibrate, and were then given a conditioning stretch of ~0.6 mN prior to determining the passive length-tension curve. To generate this curve, each artery was stretched in four 25 μm incremental steps and held for 20 s at each step, and the resistive force was measured. The 25 μm increases in length and the forces produced were used to determine the resting length-tension relationship. The arterial circumference was obtained by using Laplace's law, an equation which gives the circumference the vessel would have at 100 mmHg (L_{100}) from the exponential curve fit of tension *vs* circumference. The calculated values of this parameter did not differ between vessels from +/? and cp/cp rats (1040 ± 26 and 1106 ± 24 μM, respectively). The concentration-response curve was obtained at 0.8 L_{100}, a point which provides maximum active force generation with minimum passive tension.

Experimental design

The study was divided into three different protocols. In the first, the effect of incubation in the presence of $CuSO_4$ was studied using arteries from four male cp/cp and five male +/? rats. Cumulative concentrations of NA were added to the organ baths over the range of 10^{-8}–10^{-5} M and the force produced was measured. The arteries were washed three times by changes in buffer at 10 min intervals between concentration-response curve determinations. Each artery was then preconstricted with NA to 50% of its maximal constriction. Cumulative concentrations of ACh (10^{-9}–10^{-6} M) were added and the per cent relaxation was calculated. Relaxation curves in response to cumulative concentrations of sodium nitroprusside (SNP, 10^{-9}–10^{-4} M) were then determined. $CuSO_4$ was added to the baths at concentrations of 0, 0.25, 0.5, or 1.0 μM, and the relaxation curves in response to acetylcholine (ACh) were re-determined. The arterial vessels were then incubated in the respective solutions, and the observations of relaxation were repeated after 2 h with the $CuSO_4$ present. On completion of the protocol, the maximal constriction to buffer containing high potassium (125 mM) was measured. The maximum contractility did not vary between the rings from +/? and cp/cp rats (1.31 ± 0.11 and 1.51 ± 0.08 g, respectively). The reproducibility of repeated curves in these studies was determined in a set of preliminary experiments designed to test for tachyphylaxis.

A second protocol studied the effect of incubation with $CuSO_4$ without any direct effect of the presence of Cu^{2+}. Arteries from six male cp/cp and six male +/? rats were compared in a protocol identical to the first except that the relaxation responses to ACh both at baseline and after 2 h of incubation in $CuSO_4$ were determined after washing the arteries with three changes of buffer. The relaxation response to SNP was also measured after 2 h of incubation, following washing of the arteries.

A third protocol was designed to determine whether or not observations in the second protocol could be attributed to the effect of oxygen free radicals. Relaxation in response to ACh was measured in arteries from five male cp/cp and five male +/? rats. The arteries were then incubated for 2 h in buffer

containing either 1.0 μM $CuSO_4$, 1.0 μM $CuSO_4$ with superoxide dismutase (SOD, 50 u ml^{-1}), or 1.0 μM $CuSO_4$ with SOD (50 u ml^{-1}) and catalase (500 u ml^{-1}). The relaxation response to ACh was measured after washing the arteries with three changes of buffer.

Western blot analysis for superoxide dismutase

Western immunoblotting was performed for Cu-Zn SOD. For gel electrophoresis, samples were diluted by adding an equal volume of 2 × gel sample buffer (Tris-HCl 40 mM, pH 6.8, sodium dodecyl sulphate 2%, 2-mercaptoethanol 10%, glycerol 20%, and bromphenol blue 0.02%). Samples were boiled for 3 min. Equal protein (5 μg) was loaded into individual wells formed within the stacking gel (5% acrylamide in stacking gel buffer, Tris-HCl 25 mM, pH 6.8) overlaid on 12% acrylamide gels in Tris-HCl, pH 8.8, and separated by electrophoresis at 120 V for 1.25 h in an EC250 mini-gel apparatus (EC Apparatus, Holbrook, NY, U.S.A.).

Following separation, the samples were transferred onto a nylon membrane (Nylon NT, Micron Separations, Westborough, MA, U.S.A.). Prestained standards (kaleidoscope prestained standards, Bio-Rad Laboratories, Hercules, CA, U.S.A.) were included in separate lanes in each gel for identification of the approximate molecular weight of unknowns. Primary polyclonal antibody (Oxis International, Portland, OR, U.S.A.) was incubated for 2 h; secondary antibody conjugated to horseradish peroxidase (Jackson Immunoresearch, Westgrove, PA, U.S.A.) was incubated for 1 h; and ECL detection (Amersham LIFE Science, Oakville, ON, Canada) was performed. Autoradiography was performed using medical X-ray film (Fuji Photo Film, Tokyo, Japan).

Plasma glucose and insulin

Blood was collected from the rats in the normal fed state *via* left ventricular puncture and was placed into tubes containing ethylene diamine tetraacetic acid. Plasma was separated by centrifugation (6500 × *g*, 4°C for 10 min). Plasma glucose was assayed using a glucose oxidase procedure (Beckman Instruments, Brea, CA, U.S.A.). Insulin was measured by radioimmunoassay with rat insulin standards (Kabi Pharmacia Diagnostics AB, Uppsala, Sweden).

Drugs

NA, ACh, SNP, SOD, and catalase were obtained from Sigma Chemical (St. Louis, MO, U.S.A.). Stock solutions of the drugs were prepared in distilled water, except for the enzymes, which were prepared in buffer and added directly to the organ bath. All concentrations are expressed as the final concentration in the tissue bath fluid.

Statistics

The force produced was calculated as the acquired tension per dose of drug (i.e., measured tension minus baseline tension). Data from relaxation concentration-response curves were fitted to sigmoidal curves using the Prism computer program (Graphpad, San Diego, CA, U.S.A.), yielding values for the four variables of the logistic equation. Data for EC_{50} were log transformed and, together with the maximum/minimum responses, were analysed by Student's *t*-test, ANOVA, and correlation analysis, as appropriate (SPSS, v. 6.1.3, SPSS Inc., Chicago, IL, U.S.A.). A value of $P<0.05$ was considered as being significant. All data are presented as the mean ± s.e.mean and as EC_{50} values for ease of interpretation.

Results

Table 1 shows the characteristics of the animals studied. The cp/cp rats had markedly greater body weights and markedly higher non-fasting insulin levels than did the +/? rats. Both genotypes showed non-hyperglycaemic plasma glucose concentrations, which tend to be variable in this strain and were higher in the +/? rats in this study.

There was no difference in the contractile concentration response to NA of the arteries from the +/? and cp/cp rats (EC_{50} values of 1.15 ± 0.07 and 1.15 ± 0.05, respectively). Moreover, the presence of Cu^{2+} or SOD in the bath did not alter the EC_{50} to NA in either genotype. The arteries from cp/cp rats had a significantly reduced relaxation response to ACh compared to those from +/? rats, as shown by a greater EC_{50} (7.02 ± 0.98 *vs* 3.42 ± 0.97 × 10^{-8} M, $P<0.01$; Figure 1). The relaxation response to SNP was also impaired in the arteries from cp/cp rats (EC_{50} of 9.94 ± 0.81 *vs* 3.80 ± 0.92 × 10^{-8} M, $P<0.05$; Figure 1).

Figure 2 shows relaxation of the arteries in response to ACh after a 2 h incubation with $CuSO_4$. In this experiment, $CuSO_4$ was present in the organ bath when the relaxation response was measured. The presence of $CuSO_4$ at 1.0 μM caused a reduction in the maximum relaxation to ACh, from 101 ± 2.1 to 73 ± 1.8% in rings from +/? rats and from 100 ± 1.8 to 79 ± 2.3% in rings from cp/cp animals ($P<0.001$). The EC_{50} values were not significantly changed following incubation in the presence of 1.0 μM Cu^{2+}, in either genotype.

Figure 3 shows the concentration-response curves to ACh of arteries from +/? and cp/cp rats after 2 h of incubation in $CuSO_4$ and washing of the arteries to remove the $CuSO_4$ prior to measurement. Under these conditions, the maximum relaxation of arteries from cp/cp rats was less impaired by copper incubation than was that of arteries from +/? rats (74 ± 1.9 *vs* 60 ± 1.2% at 1 μM Cu^{2+}, $P=0.04$). The impairment in maximum relaxation increased with a 4 h exposure to $CuSO_4$ ($P<0.001$, data not shown). Figure 4 shows an increased response of arteries from +/? and cp/cp rats to ACh immediately following exposure to $CuSO_4$. The EC_{50} for ACh decreased from 2.28 ± 0.20 to 0.71 ± 0.09 × 10^{-8} M for rings from +/? rats and from 7.31 ± 0.41 to

Table 1 Body weight and plasma insulin and glucose concentrations in 6-month-old male JCR:LA-cp rats

	+/? Rats	*cp/cp Rats*
Body weight (g)	395 ± 6.0	639 ± 7.7**
Plasma glucose (mg dl^{-1})	192 ± 11	147 ± 14*
Plasma insulin (mu l^{-1})	72 ± 9.4	568 ± 110**

Values are mean ± s.e.mean, 10 animals in each group. $P<0.01$ *vs* +/?, **$P<0.001$.

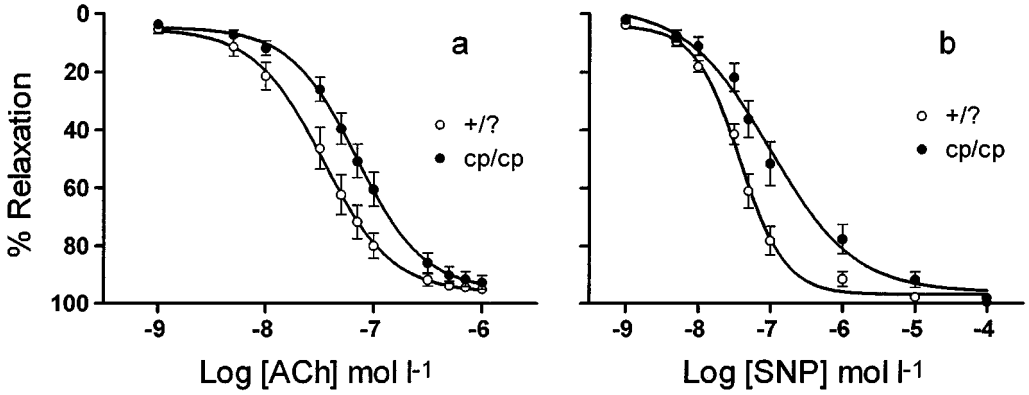

Figure 1 Relaxation of mesenteric artery segments precontracted with noradrenaline in response to (a) acetylcholine (ACh) and (b) sodium nitroprusside (SNP). Values are mean ± s.e.mean, 10 rats in each group. The EC_{50} was significantly greater for cp/cp rats compared to +/? rats for ACh ($P<0.01$) and SNP ($P<0.05$).

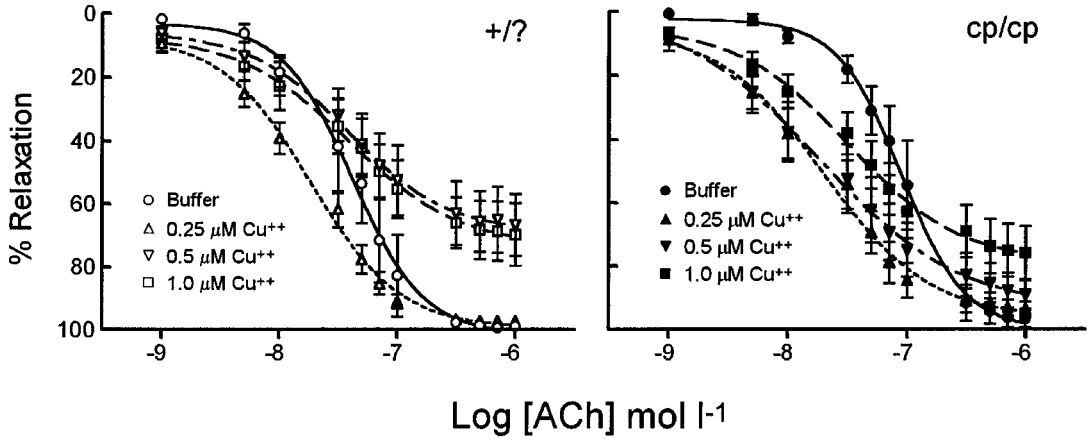

Figure 2 Relaxation of noradrenaline-precontracted mesenteric artery segments in response to acetylcholine (ACh) after 2 h of incubation in the presence of $CuSO_4$. Values are mean ± s.e.mean, five rats in each group. The maximal relaxation was significantly decreased after incubation with 1.0 μM $CuSO_4$ in both genotypes and at 0.5 μM in the rings from +/? rats ($P<0.001$). There were no significant changes in the EC_{50} following incubation in the presence of $CuSO_4$ in either genotype.

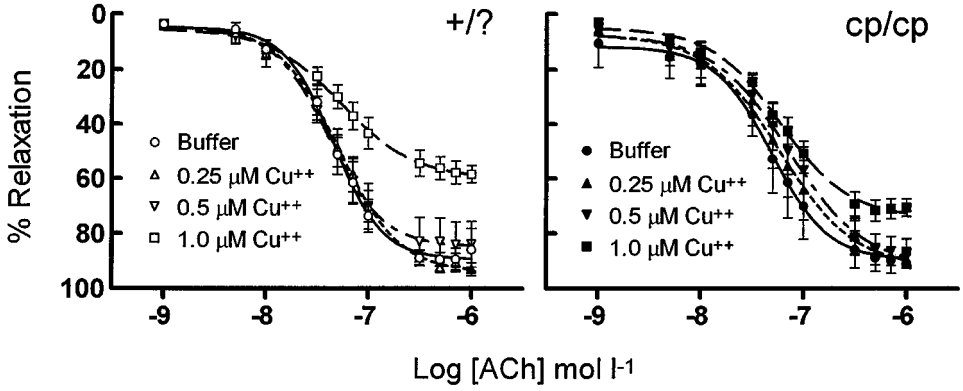

Figure 3 Relaxation in response to acetylcholine (ACh) of mesenteric artery segments incubated for 2 h in the presence of $CuSO_4$, washed to remove the Cu^{2+}, and precontracted with noradrenaline. Values are mean ± s.e.mean, six rats in each group. Maximum relaxation was reduced at the higher concentrations of $CuSO_4$ in both genotypes ($P<0.001$).

$0.96 \pm 0.12 \times 10^{-8}$ M for rings from cp/cp rats ($P<0.001$). The EC_{50} for ACh showed a significant dependence on copper concentration ($P<0.001$) when data were normalized to the initial or baseline EC_{50} for ACh. However, there was no significant difference between genotypes in the dependence of the EC_{50} for ACh on exposure to copper.

There was a significant effect of Cu^{2+} incubation in the maximum relaxation in response to SNP following Cu^{2+} incubation (reduction from 96 ± 2 to $76\pm4\%$ in rings from +/? rats and from 93 ± 3 to $81\pm3\%$ for rings from cp/cp rats, $P<0.001$; Figure 5). There were also significant increases in the EC_{50} for SNP following incubation in the presence of Cu^{2+} (from 4.27 ± 0.42 to $75.5\pm26.7\times10^{-8}$ M for rings from +/? rats and from 6.65 ± 1.29 to $101\pm37\times10^{-8}$ M for rings from cp/cp rats, $P<0.005$ and $P<0.02$, respectively).

Arteries incubated with Cu-Zn SOD and catalase were completely protected from the impairment of ACh-mediated relaxation caused by incubation with 1.0 μM $CuSO_4$, with the dose-response curves being indistinguishable from those of untreated rings ($P=0.001$ *vs* untreated, both genotypes; Figures 3 and 6). SOD without catalase gave no protection from the effect of incubation with 1.0 μM $CuSO_4$. Protein levels of Cu-Zn SOD in cp/cp rat aorta were not significantly different from those of +/? aorta, as shown by Western blotting (Figure 7).

Discussion

This work was carried out on 6-month-old animals in order to study vascular wall function at an age when the insulin-resistant state is fully established and arteries have been exposed to hyperinsulinaemia for some time. Compared with the mesenteric arteries of +/? animals, those of cp/cp rats had impaired relaxation in response to ACh as well as to SNP, similar to our previous findings (O'Brien *et al*., 1999). Arterial responses to ACh and SNP were not correlated, indicating that cp/cp rats have an impaired endothelial function in addition to an impaired smooth muscle response to NO. We have also found the vascular smooth muscle cells of the cp/cp rat to be hyperproliferative and migratory (Absher *et al*., 1997; Richardson *et al*., 1998), with both effects being insulin dependent (Absher *et al*., 1998). Impaired endothelial function in both obese, insulin-resistant humans (Steinberg *et al*., 1996) and those with non-insulin-dependent diabetes mellitus (Watts *et al*., 1996) has also previously been reported.

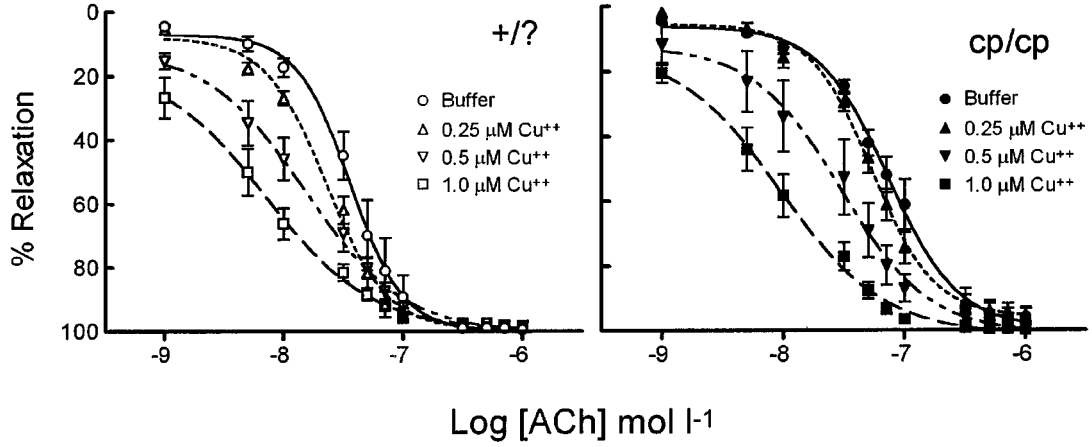

Figure 4 Relaxation of noradrenaline-precontracted mesenteric artery segments in response to acetylcholine (ACh) in the presence of $CuSO_4$, but without a period of incubation. Values are mean$\pm$s.e.mean, six rats in each group. The reduction in maximum relaxation was less in cp/cp rat arteries than in those from +/? rats ($P=0.04$).

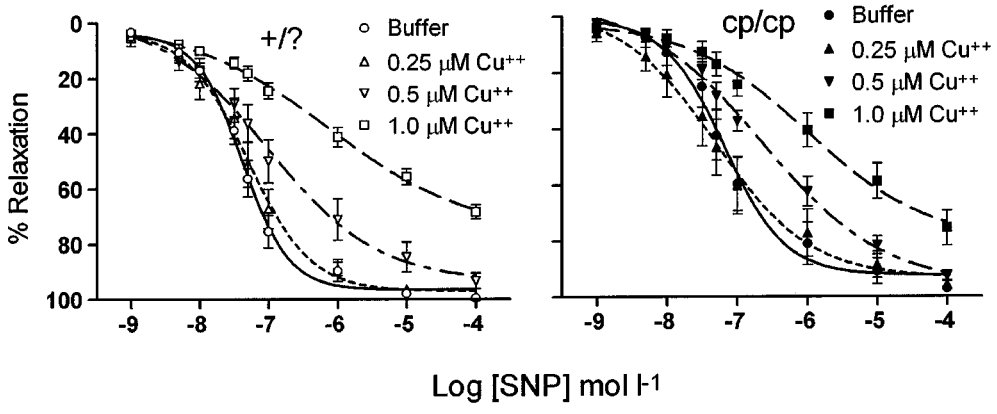

Figure 5 Relaxation in response to sodium nitroprusside (SNP) of mesenteric artery segments treated as in Figure 4. Values are mean$\pm$s.e.mean, six rats in each group. The maximum relaxation was impaired by $CuSO_4$ incubation in both genotypes ($P<0.001$) and the EC_{50} was increased ($P<0.005$ for +/? rats and $P<0.02$ for cp/cp rats).

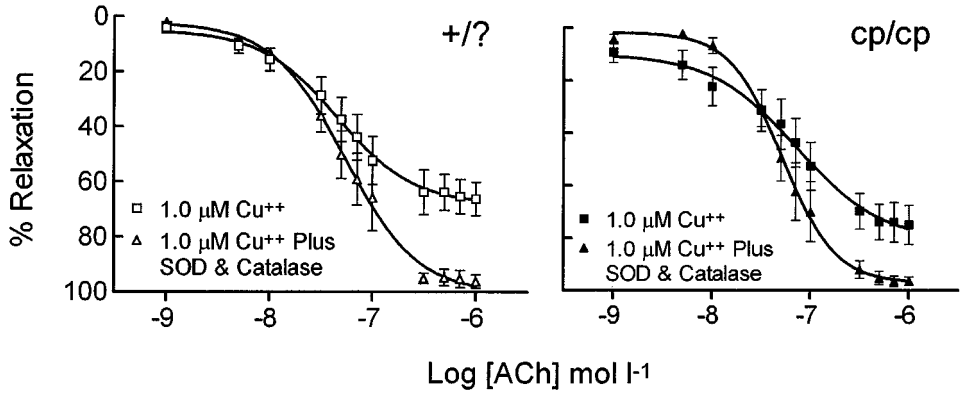

Figure 6 Relaxation in response to acetylcholine (ACh) of mesenteric artery segments incubated for 2 h in the presence of 1.0 μM $CuSO_4$ plus superoxide dismutase and catalase. Values are mean$\pm$s.e.mean, five rats in each group. The significant improvement in ACh-mediated relaxation ($P<0.001$) restored relaxation to levels similar to those of control arteries (see Figure 3).

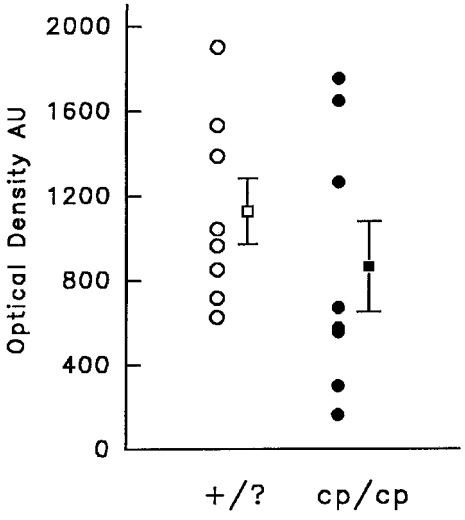

Figure 7 Scatter plot of the density of Western blot band for superoxide dismutase from rat aorta. The values of 1128 ± 156 and 864 ± 215, shown as squares and error bars (mean$\pm$s.e.mean), respectively, are not significantly different.

The roles of copper and oxidative stress on arterial contraction and relaxation have not been extensively studied. Rat aortic rings have been reported as showing enhanced relaxation in response to SNP and the calcium ionophore, A23187, when high concentrations of Cu^{2+} (100 μM), but not of Fe^{2+} (100 μM), were present (Plane *et al*., 1997). We have found an immediate enhanced relaxant response to ACh at much lower concentrations of Cu^{2+} ($\leqslant$1.0 μM). Incubation of the arterial rings for 2 h in the presence of Cu^{2+} (giving an oxidative exposure) resulted in impaired relaxation. The impairment was less severe in the arteries from cp/cp than in +/? rats once the Cu^{2+} had been removed by post-incubation washing.

We did not observe any differential effects between cp/cp and +/? rats, either immediately or after incubation, when Cu^{2+} was present. These results suggest that Cu^{2+} has a dual action on vascular function, whereby function is enhanced on initial Cu^{2+} exposure, perhaps by a direct cellular effect of Cu^{2+} itself, and subsequently is impaired over time as oxidation proceeds. Thus, when arteries were incubated with Cu^{2+} and thoroughly washed with buffer prior to the addition of vasorelaxants, the direct relaxant effect of Cu^{2+} was removed. Under these conditions, the severity of the relaxation impairment differed between cp/cp and +/? rats, with the arteries from cp/cp rats being less affected than those from +/? rats. The impairment in maximum relaxation was evident primarily at the highest concentration of Cu^{2+} used (1 μM). The relaxation in response to SNP showed a similar pattern of impairment following incubation in Cu^{2+} and removal of the Cu^{2+}. The impairment in maximum relaxation was somewhat greater for SNP and was accompanied by quite large increases in EC_{50}. Feelisch *et al*. (1999) have reported that SNP may cause relaxant effects through indirect pathways and these may involve direct activation of soluble guanylate cyclase (Tseng *et al*., 2000). Thus, the greater impairment of the response to SNP may reflect dysfunction in non-NO-mediated pathways. These changes suggest direct oxidative damage to the vascular smooth muscle cells.

Our results are consistent with reports that Cu^{2+} has a direct effect on endothelial cells in which endothelial NO synthase is activated (Kishimoto *et al*., 1996), and this may counteract impairment due to longer-term exposure to Cu^{2+}, which we suggest to be due to oxidative stress. Endothelial cells have been reported to prevent the accumulation of lipid peroxides in the presence of Cu^{2+} at concentrations up to 0.75 μM, whereas at higher concentrations lipid peroxidation in endothelial cells was enhanced (Smalley *et al*., 1997). Our findings are consistent with this notion. The arteries from cp/cp rats appeared to have greater resistance to oxidative stress, possibly as an adaptation to persistent oxidative stress or as a reduced propensity to greater lipid peroxidation at higher concentrations of Cu^{2+}.

Endothelium-dependent relaxation has been reported to be impaired by oxidative stress (Dudgeon *et al*., 1998). The physiological antioxidant defence is multi-tiered and includes both dietary and endogenously produced lipid- and water-soluble antioxidants, as well as antioxidant enzymes, principal amongst which is Cu-Zn SOD. The inactivation of Cu-Zn SOD enhances oxidative stress in arteries and results in impaired ACh-mediated relaxation (Mackenzie & Martin, 1998). We have shown that incubating arteries with the combination of Cu-Zn SOD and catalase completely

prevented the impairment in ACh-mediated relaxation caused by incubation with 1 μM Cu^{2+}, whereas incubation in Cu-Zn SOD without catalase did not prevent the impairment. This indicates that the impairment in function caused by incubation with Cu^{2+} was due to oxidative stress. Cu-Zn SOD catalyses the dismutation of two superoxide radical ions to form hydrogen peroxide (H_2O_2); catalase further reduces the H_2O_2 to water. Our results indicate that H_2O_2 and/or the potent oxidant $OH^{\bullet}$ (produced by the Fenton reaction) caused the impaired vascular function seen in the Cu^{2+}-treated arteries. The absence of any significant differences between Cu-Zn SOD concentrations in the aortae of cp/cp and +/? rats suggests that the reduced sensitivity of the cp/cp rat to oxidative damage is not due to greater SOD capacity, at least not of the Cn-Zn isoform. However, this does not exclude a compensatory upregulation of other isoforms of SOD such as ecSOD, as reported by Fukai *et al*. (2000). The role of SOD in the potentiation or inhibition of atherogenesis is not clear and there is evidence to suggest that in some circumstances Cu-Zn SOD may be deleterious (Tribble *et al*., 1997).

The vasorelaxant effect of Cu^{2+} seen in the JCR : LA-cp rat is evident at much lower concentrations than has been reported in other rat strains (Demura *et al*., 1998). Furthermore, when the immediate vasorelaxant effect of Cu^{2+} was removed, a less severe impairment in ACh-mediated relaxation was evident in the arteries from cp/cp rats than in those from lean +/? rats. This indicates that the obese, insulin-resistant status is associated with some protection against the effects of oxidative stress on the endothelium, possibly through an adaptation to chronic oxidative stress. In contrast, the direct inhibiting effect of oxidation on the vascular smooth muscle cells, reflected in the impaired SNP-mediated relaxation, does not appear to be modulated by insulin resistance. It is also possible that insulin resistance may be, at least in part, an adaptation to oxidative stress. For example, Rudich *et al*. (1997) reported that adipocytes were less responsive to insulin after exposure to oxidative stress. Impaired relaxation in response to glycerol trinitrate, but not to ACh, in the less insulin-resistant fatty Zucker rat has been found to be restored by dietary antioxidants (Laight *et al*., 1998). The role of oxidative stress in the physiology of obesity and insulin resistance may therefore be part of a feedback loop and may contribute, both directly and indirectly, to vasculopathy and cardiovascular disease.

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