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Inhibition of endogenous nitric oxide in the heart enhances matrix metalloproteinase-2 release

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1 Matrix metalloproteinase (MMP) activity is upregulated in pathologies such as atherosclerosis during which endogenous nitric oxide (NO) biosynthesis is reduced. Diminished levels of NO, an antioxidant species, may result in higher oxidative stress. Oxidants are capable of activating MMPs from their zymogen forms. We examined whether basal biosynthesis of NO in the coronary circulation regulates MMP-2 activity.

2 In isolated rat hearts perfused with Krebs–Henseleit buffer at a constant flow of 10 ml min^{-1}, we measured the release of MMP-2 into the coronary effluent by gelatin zymography. The main gelatinolytic activity of 72-kDa corresponds to MMP-2. Infusion of the NO synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) concentration dependently increased coronary perfusion pressure (CPP) (by 48 ± 11 mmHg with 100 μM) and enhanced the release of the 72-kDa MMP-2 in the effluent. Coinfusion of the NO donor *S*-nitroso-*N*-acetyl-D,L-penicillamine (SNAP, 1 μM) with L-NAME abolished both the increase in CPP and the enhanced MMP-2 release.

3 The thromboxane A_2 mimetic U46619 increased CPP to the same extent as L-NAME without increasing 72-kDa activity in the effluent, suggesting that MMP-2 release is not caused simply by enhanced perfusion pressure.

4 Infusion of either L-NAME or U46619 did not significantly enhance LDH release.

5 L-NAME infusion concentration dependently increased the level of lipid hydroperoxides in homogenates prepared from the perfused hearts. Coinfusion of SNAP prevented this increase.

6 These data reveal another cytoprotective mechanism of endogenous NO biosynthesis in the heart, the inhibition of MMP-2 release.
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Abbreviations: CPP, coronary perfusion pressure; LDH, lactate dehydrogenase; L-NAME, N^G-nitro-L-arginine methyl ester; LVDP, left ventricular developed pressure; MMP, matrix metalloproteinase; NO, nitric oxide; NOS, NO synthase; RPP, product of heart rate and left ventricular developed pressure; SNAP, *S*-nitroso-*N*-acetyl-D,L-penicillamine

Introduction

Nitric oxide (NO) is an important biological messenger with a variety of roles in the cardiovascular system. Its biological functions in the heart include vasodilation, inhibition of platelet and neutrophil actions, and the modulation of cardiac contractile function and oxygen consumption (see Paulus & Shah, 1999 for a review). Moreover, endogenous NO is an important antioxidant defense in the body. Although NO combines with superoxide at a diffusion-limit rate to form the cytotoxic molecule peroxynitrite (Beckman *et al*., 1990), NO itself has been shown to counteract peroxynitrite-mediated cyotoxicity (Yasmin *et al*., 1997; Trostchansky *et al*., 2001). Moreover, NO is able to prevent ischemia–reperfusion damage in the heart (Johnson *et al*., 1991; Morikawa *et al*., 1992). One mechanism by which this occurs is by inhibiting the propagation of free radical chain reactions in the lipid membrane (Rubbo *et al*., 1994; 2000; Goss *et al*., 1997). Deficiency or enhanced degradation of endogenous NO may lead to greater oxidative stress, as observed in many pathological conditions such as atherosclerosis (Ludmer *et al*., 1986), essential hypertension (Calver *et al*., 1992), diabetes (Amado *et al*., 1993), coronary artery disease (De Belder & Radomski, 1994) and heart failure (Vallance & Chan, 2001).

MMPs are a family of at least 25 zinc-containing endopeptidases, which are best recognized for their ability to degrade components of the extracellular matrix (Woessner, 1998). Activation of their zymogen forms is a very important step in the regulation of their activity. Breakage of the cysteinyl sulfhydryl bond between a cysteine residue of the propeptide and the Zn^{2+} catalytic center is necessary for the activation of the zymogen form (Nagase, 1997). This can be achieved either by cleavage of the propeptide by other proteases including MMPs, resulting in an active enzyme with 8–10 kDa lower mass, or by conformational changes induced by denaturing agents such as sodium dodecyl sulfate (SDS) (Van Wart & Birkedal-Hansen, 1990). More recently, oxidant

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stress molecules like peroxynitrite have been shown to activate MMPs without proteolytic removal of the autoinhibitory propeptide domain. This mechanism includes a novel S-glutathiolation of the critical cysteine residue in the inhibitory propeptide domain of MMP, resulting in the activation of the full length enzyme (Okamoto *et al*., 2001).

In previous studies, we have found that there is a basal release of MMP-2 into the coronary effluent of aerobically perfused isolated rat hearts. The release of MMP-2 is rapidly enhanced in the first minute of reperfusion following ischemia (a condition of enhanced oxidative stress) and this contributes to the immediate impairment in myocardial contractile function (Cheung *et al*., 2000b). Direct infusion of peroxynitrite into isolated rat hearts caused a rapid increase of MMP-2 release into the coronary effluent, which preceded the onset of the decline in myocardial contractile function. Enhancing the antioxidant defense with infusion of glutathione or inhibiting MMP activity could prevent the detrimental effects of peroxynitrite. In contrast, the NO donor SNAP did not enhance MMP-2 release (Wang *et al*., 2002a). Although exogenous NO has been shown to inhibit the release of MMPs from platelets (Sawicki *et al*., 1997), tumor cells (Jurasz *et al*., 2001) and cartilage (Cao *et al*., 1998), the interaction of NO and MMP-2 in the intact coronary circulation remains unknown. We hypothesized that basal NO generation in the coronary vasculature may regulate MMP-2 release *via* its antioxidant properties and tested this using an NO synthase (NOS) inhibitor strategy in isolated perfused rat hearts.

Methods

This investigation conforms to the Guide to the Care and Use of Experimental Animals published by the Canadian Council on Animal Care (revised 1993).

Materials

The supernatant from phorbol ester-activated human fibroblast HT-1080 cells (American Type Culture Collection, Rockville, MD, U.S.A.), which contains large amounts of MMP-2 and MMP-9, was used as a standard. All other reagents were purchased from Sigma-Aldrich, Oakville, Ontario, Canada.

Heart preparation and perfusion

Male Sprague–Dawley rats (250–300 g) were used for the experiments. Hearts were rapidly excised from pentobarbital-anesthetized rats and briefly rinsed by immersion into ice-cold Krebs–Henseleit solution. They were perfused *via* the aorta at a constant flow of 10 ml min^{-1} by means of a peristaltic pump (Buchler Instruments Inc., Fort Lee, NJ, U.S.A.) with Krebs–Henseleit buffer at 37°C. The composition of the buffer was (in mM): NaCl (118), KCl (4.7), KH_2PO_4 (1.2), $MgSO_4$ (1.2), $CaCl_2$ (3.0), $NaHCO_3$ (25), glucose (11), EDTA (0.5) and it was continuously gassed with 95% O_2/5% CO_2 (pH 7.4).

Spontaneously beating hearts were used in all experiments. A water-filled latex balloon connected to a pressure transducer was inserted into the left ventricle through an incision in the left atrium and through the mitral valve and the volume was adjusted to achieve an end diastolic pressure of 8–12 mmHg. A transducer was placed in the infusion line close to the heart to monitor coronary perfusion pressure (CPP). A water-jacketed glass chamber was positioned around the heart to maintain its temperature at 37°C. CPP, heart rate and left ventricular pressure were monitored on an IBM PC compatible computer using an MP100 system (BIOPAC system Inc., Santa Barbara, CA, U.S.A.). Left ventricular developed pressure (LVDP) was calculated as the difference between systolic and diastolic pressures of the left ventricular pressure trace. The rate–pressure product (RPP) was calculated as the product of heart rate and LVDP. Stock solutions of various reagents were infused into the heart *via* a side-port proximal to the aortic cannula at a constant rate of 0.1 ml min^{-1} by a Gilson mini pump (Minipuls 3, Villiers Le Bel, France).

The intervals between thoracotomy and attachment of the heart to the perfusion system and between thoracotomy and beginning of stabilization period were less than 1 and 5 min, respectively. Using this perfusion protocol, the hearts maintained a steady state of coronary flow, heart rate and LVDP for longer than 65 min after stabilization, verifying previous results from this laboratory (Cheung & Schulz, 1997).

Infusion of NOS inhibitors

Following 20 min of perfusion (stabilization period), the thromboxane mimetic U46619 was infused at a concentration sufficient to increase the CPP ~20 mmHg from a baseline of 40 mmHg (9.1 ± 2.2 nM). The infusion of U46619 was maintained throughout the duration of the perfusion. After 15 min of U46619 infusion, when the increase in CPP reached a plateau in all hearts, either L-NAME (10 or 100 μM) or its vehicle was infused into the hearts for 15 min followed by a 15 min washout period. Hearts were perfused for a total duration of 65 min and their mechanical function was recorded. SNAP (1 μM) was coinfused with 100 μM L-NAME for the same duration into a separate group of hearts. A measure of 6 ml of coronary effluent was collected immediately before, and 15 min after beginning the infusion of L-NAME, and again at the end of washout period. Coronary effluent samples were immediately placed on ice and processed on the same day, as described below. At the end of the perfusion, hearts were freeze-clamped with tongs cooled to the temperature of liquid nitrogen and stored at −80°C.

A higher concentration of U46619 (37.8 ± 14.5 nM) was infused as a control to account for vasoconstrictor action of the L-NAME into a separate group of hearts. Coronary effluent samples were collected as above.

Protein concentration of the coronary effluent

The 6 ml coronary effluent samples were concentrated in Centricon-30 concentrating vessels (Amicon Inc., Beverly, MA, U.S.A.). The final volume of concentrate was measured by gravimetry, and adjusted in order to have same final volume for each sample. Protein concentration in the concentrate was determined by bicinchoninic acid assay using bovine serum albumin as standard. The protein concentration in the coronary effluent was calculated and expressed in μg ml^{-1}.

Measurement of MMPs by gelatin zymography

Gelatin zymography was performed as described previously (Cheung *et al*., 2000a). Briefly, concentrated coronary effluent

samples were mixed with nonreducing sample loading buffer, and applied to 8% polyacrylamide gels copolymerized with 2 mg ml^{-1} gelatin. In all, 1–2 μg of effluent concentrate was loaded in each lane. After electrophoresis, gels were rinsed in 2.5% Triton X-100 (3 × 20 min) to remove SDS. Then, the gels were washed twice in incubation buffer for 20 min each at room temperature. The composition of the incubation buffer was (in mM): Tris-HCl (50), $CaCl_2$ (5), NaCl (150) and 0.05% NaN_3. The gels were then kept in incubation buffer at 37°C overnight. After incubation, gels were stained in staining solution (2% Coomassie Brilliant blue G, 25% methanol, 10% acetic acid) for 2 h and then destained twice for 30 min each in destaining solution (2% methanol, 4% acetic acid). Zymograms were scanned using an HP 6100 scanner (Hewlett-Packard), and the band intensities were analyzed by Sigmagel software (version 1.0, Jandel Scientific). Conditioned culture media from a human fibrosarcoma cell line HT-1080 (American Type Culture, Rockville, MD, U.S.A.), which contains large amounts of MMP-2, was used as a standard in gelatin zymography. MMP activities were expressed as a specific activity per microgram protein in the coronary effluent.

Lipid hydroperoxide assay

As a measure of oxidative stress, lipid hydroperoxide content of heart tissue was determined using a commercial kit based on a colorimetric assay, as described previously (Cheung *et al*., 2000a). After deproteinization and extraction of the samples with methanol and chloroform, the absorbance at 500 nm was determined in a spectrophotometer using a quartz 96-well plate. The level of lipid hydroperoxide was expressed as pmol mg protein^{-1}.

Lactate dehydrogenase (LDH) measurement

LDH activity was determined in the concentrated coronary effluents as a marker of cardiac damage. A commercially available kit was used (Sigma-Aldrich, Oakville, Ontario, Canada) and the formation of a UV-absorbing product was monitored by spectrophotometric analysis.

Data analysis

Data were expressed as mean ± s.e.m. One-way ANOVA with Student–Neuman–Keuls *post hoc* test was used for statistical analysis. A value of P less than 0.05 was considered statistically significant.

Results

Effect of L-NAME on coronary vascular tone and MMP-2 release into coronary effluent

Infusion of L-NAME (10 or 100 μM) for 15 min increased coronary vascular tone in a concentration-dependent manner as demonstrated by an increase in the CPP by 19.4 ± 8.3 and 47.5 ± 10.7 mmHg, respectively (Figure 1a, Table 1). Infusion of L-NAME at these concentrations did not have any effect on cardiac mechanical function (Table 1). In accordance with our previous reports (Cheung *et al*., 2000b; Wang *et al*., 2002a), the only MMP activity readily detectable by gelatin zymography

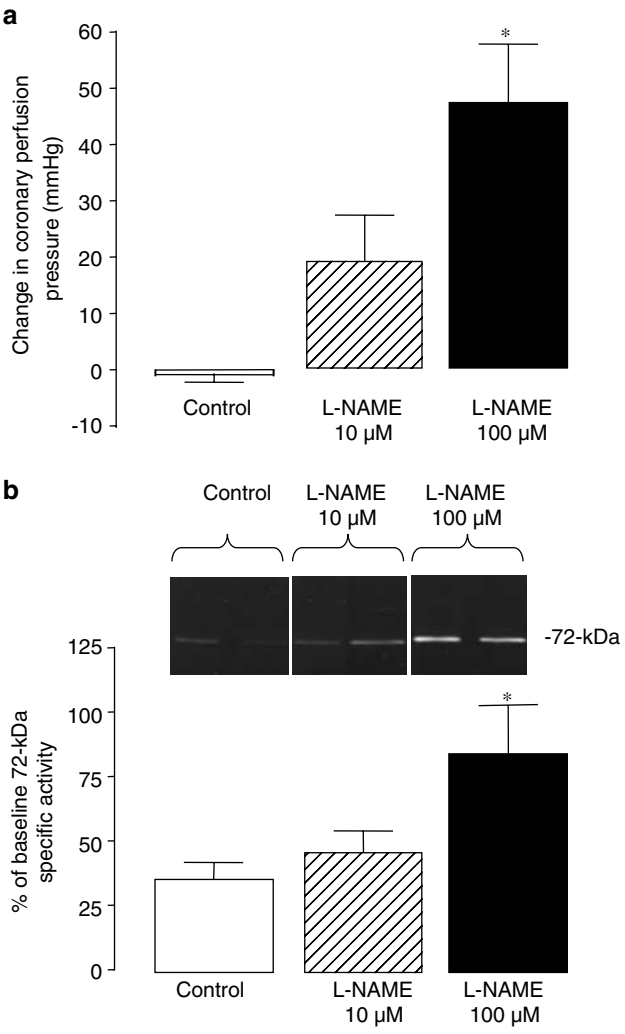

Figure 1 Effects of L-NAME (10 and 100 μM) on CPP (a) and MMP-2 release into the coronary effluent (b) from isolated perfused rat hearts. Inset shows representative zymograms of coronary effluent samples obtained from two control, two 10 μM L-NAME and two 100 μM L-NAME-infused hearts. *$P<0.05$ vs control group, $n=5$–8 per group.

in the perfusate was 72-kDa MMP-2. A 62-kDa MMP-2 was seen in some perfusate samples when a longer incubation time during zymography was used. Therefore, in this study, we focused on the release of 72-kDa MMP-2 from the heart. The baseline MMP-2 activity, defined as 100%, was determined at 35 min perfusion, the time point immediately before the 15 min infusion of L-NAME. During the perfusion of control hearts, the release of MMP-2 gradually declined by 50 min to 36.2 ± 7.4% of the baseline level. Infusion of L-NAME concentration dependently increased MMP-2 release (to 46.5 ± 8.7 and 83.7 ± 18.8% of baseline in 10 and 100 μM L-NAME groups, respectively, Figure 1b). The effect of L-NAME treatment was not due to changes in general total protein release into the coronary effluent ($P>0.05$, data not shown).

Effect of coinfusion of SNAP with L-NAME on coronary vascular tone and MMP-2 release into coronary effluent

Replenishing basal NO levels by the coinfusion of 1 μM SNAP with L-NAME (100 μM) for 15 min not only abolished the

Table 1 Effects of treatments on heart rate, LVDP and CPP in isolated perfused rat hearts

	Heart rate (beats min^{-1})	*LVDP* (mmHg)	*CPP* (mmHg)
Control ($n=8$)	295±14	76.8±9.1	58.5±2.8
L-NAME 100 μM ($n=8$)	276±13	80.9±6.7	110.5±11.4*
L-NAME 100 μM + SNAP 1 μM ($n=6$)	283±13	73.5±6.1	56.5±5.7†

Data were recorded at 50 min perfusion time, at the end of 15 min infusion of drugs.
*$P<0.05$ vs control, $^{\dagger}P<0.05$ vs L-NAME group by one-way ANOVA with Student–Neuman–Keuls *post hoc* test.

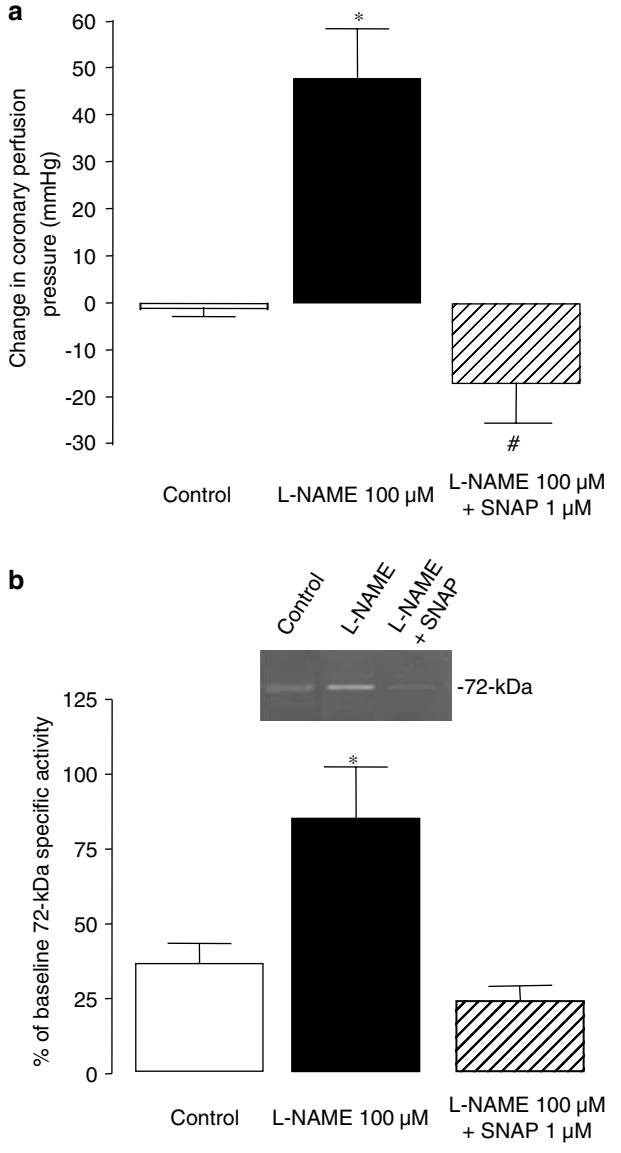

Figure 2 Effects of coinfusion of SNAP (1 μM) with 100 μM L-NAME on CPP (a) and MMP-2 release into coronary effluent (b). Inset is a representative zymogram from coronary effluent samples. *$P<0.05$ vs control group and $^{\#}P<0.05$ vs L-NAME group, $n=6$–8 in each group.

increase in CPP but also prevented the enhanced release of MMP-2 (Figure 2).

Effect of U46619 on coronary vascular tone and MMP-2 release

Infusion of a higher concentration of U46619 to induce a similar increase in CPP as that of 100 μM L-NAME did not alter the level of MMP-2 in the coronary effluent (Figure 3).

Effect of treatments on lipid hydroperoxide level in heart tissue

Infusion of L-NAME concentration dependently increased the level of lipid hydroperoxides in the myocardium (Figure 4), which became statistically significant at 100 μM. The effect of L-NAME was abolished by coinfusion of 1 μM SNAP.

Effect of treatments on LDH release from hearts

To determine whether L-NAME could cause a general damage of the cardiac tissue, we measured the release of LDH in the coronary effluent. Infusion of U46619 alone (9.1±2.2 nM) ($n=3$) or of U46619 in the presence of 100 μM L-NAME ($n=3$) resulted in a similar, not statistically different release of LDH (3.59±0.75 vs 4.31±0.82 U l^{-1}, $P>0.05$).

Discussion

Here we report that the basal generation of NO regulates the release of MMP-2 in the coronary vasculature. In many cardiac pathologies, including reduced coronary vasodilator function, either reduced basal NO biosynthesis or its enhanced degradation have been documented. We show here that reducing endogenous NO biosynthesis with L-NAME causes an increase of oxidative stress in the heart, which in turn enhances the release of MMP-2. This study provides the first evidence of an important interaction between NO biosynthesis and MMP-2 release in the heart.

NO generated by the coronary vasculature serves as one of the antioxidant defenses in the heart. Several findings support this hypothesis. NO donors were able to reduce ischemia–reperfusion damage in the heart, an insult associated with enhanced oxidative stress. Infusion of authentic NO decreased myocardial infarct size in cats after reperfusion following occlusion of the left anterior descending artery (Johnson *et al*., 1991), whereas the NO releasing nitrosothiol SNAP at subvasodilatory concentration prevented myocardial ischemia–reperfusion in isolated rat hearts (Yasmin *et al*., 1997). After occluding the middle cerebral artery, infusion of the substrate for NO biosynthesis, L-arginine, reduced cerebral infarct size in spontaneously hypertensive rats (Morikawa *et al*., 1992). Moreover, Wink *et al*. (1993) demonstrated that NO directly protected both cultured lung fibroblasts and mesencephalic cells from cytotoxicity induced by the oxidative stress of hydrogen peroxide.

Whether removal of basal NO causes an oxidative stress in the intact heart is not known. In this study, we infused an NOS inhibitor, L-NAME, into isolated rat hearts to reduce the basal generation of NO. As described previously, infusion of L-NAME concentration dependently increased coronary

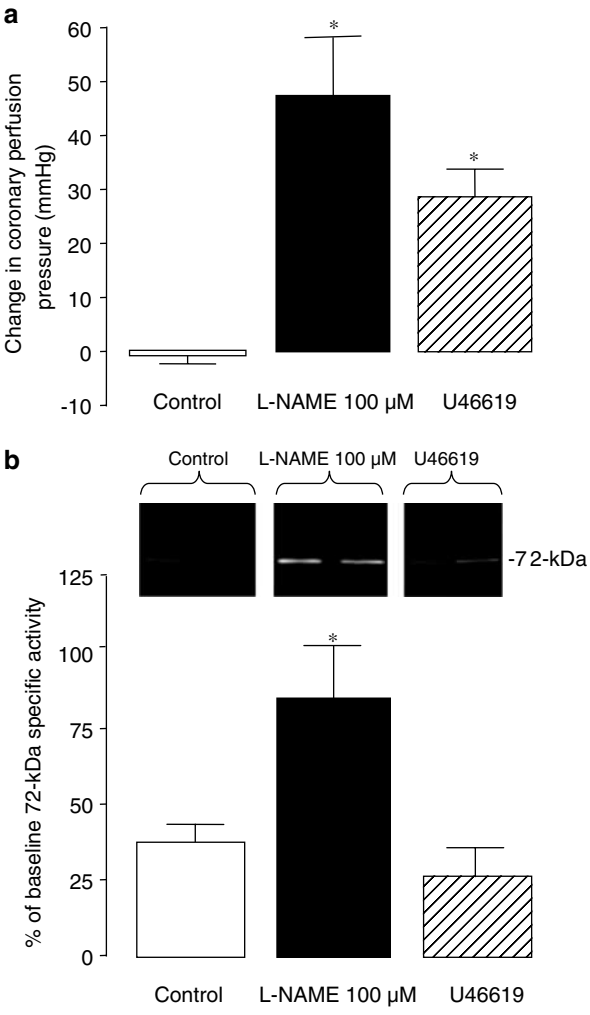

Figure 3 Effect of U46619 infusion on changes of CPP (a) and MMP-2 release into coronary effluent (b) in comparison to that of 100 μM L-NAME in isolated perfused rat hearts. U46619 was infused at a concentration to give a statistically equivalent increase in CPP (37.8 ± 14.5 nM). Inset shows representative zymograms of coronary effluent samples obtained from two control, two 100 μM L-NAME and two U46619-infused hearts. $^{*}P<0.05$ vs control group, $n=8$.

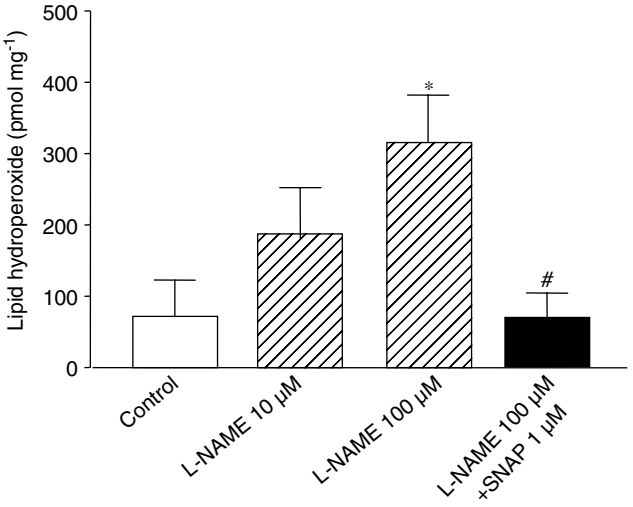

Figure 4 Lipid hydroperoxide levels in tissues from control, 10 μM L-NAME, 100 μM L-NAME and 100 μM L-NAME + 1 μM SNAP-infused hearts. $^{*}P<0.05$ vs control group and $^{\#}P<0.05$ vs L-NAME 100 μM group, $n=5$.

vascular tone (Smith *et al*., 1992). Infusion of L-NAME also increased the level of lipid hydroperoxides in the myocardium, an index of enhanced oxidative stress. Coinfusion of SNAP with L-NAME not only restored coronary vascular tone but also reduced the lipid hydroperoxide levels in the heart. The lack of effect of raising CPP with U46619 to an equivalent level to that of L-NAME shows that the release of MMP-2 activity in response to the NOS inhibitor is not related simply to enhanced perfusion pressure. The release of MMP-2 is also not due to general, *in vitro* damage to the heart, as LDH release was not different from that seen in hearts infused with U46619. Taken together, these observations show that blocking NO generation causes an increased oxidative stress in the heart.

Activation of MMPs can be achieved either by proteolytic cleavage of an inhibitory propeptide domain or by chemically induced conformational changes of the protein to expose the zinc atom in its catalytic core. Peroxynitrite was shown to induce conformational change and therefore activation of the zymogen form of MMPs without a loss of the propeptide domain (Okamoto *et al*., 1997; 2001). Indeed, infusion of peroxynitrite into isolated rat hearts increased the release of 72-kDa proteolytic MMP-2 protein in the coronary effluent (Wang *et al*., 2002a) prior to the reduction in contractile function of the heart. In the present study, infusion of L-NAME increased not only lipid hydroperoxide levels but also the release of MMP-2, suggesting that enhanced oxidative stress in the presence of the NOS inhibitor might induce the release of MMP-2. The prevention of both the increase in lipid hydroperoxides and the enhanced MMP-2 release by replenishing the loss of endogenous NO with SNAP further supports this notion.

In this study, we have no direct evidence for the cellular source of MMP-2 release in the coronary effluent. Fibroblasts have been described as a source of matrix-degrading proteases in the heart (Cleutjens *et al*., 1995; Siwik *et al*., 2001); however, MMP-2 is also expressed in cardiomyocytes (Coker *et al*., 1999), and we have shown that MMP-2 is present in the sarcomeres, where it colocalizes with troponin I and the thin myofilaments (Wang *et al*., 2002b). Another possible source for MMP-2 could be the coronary vasculature, where MMP-2 expression has been observed both at the level of endothelial cells and of vascular smooth muscle cells (Gavin *et al*., 2003; Donnini *et al*., 2004). Interestingly, NO donors or upregulation of eNOS expression in endothelial cells was shown to inhibit MMP-2 expression at the transcriptional level (Chen & Wang, 2004), but whether this occurs as a result of basal NO biosynthesis is unknown.

Limitations of the study

In the present study, we focused our attention only on the release of MMP-2 in response to inhibition of NO biosynthesis in the isolated hearts. Our choice was dictated by the fact that in the absence of blood components (a major source of MMP-8 and MMP-9), MMP-2 is the main gelatinolytic enzyme detected in the isolated perfused heart (Murphy *et al*., 1982; Mainardi *et al*., 1984; Cheung *et al*., 2000b). We cannot exclude that other MMPs may be modulated in a similar

manner, given their analogies with MMP-2 structure. MMP-1, MMP-2, MMP-8 and MMP-9 have been shown to be activated by oxidants including peroxynitrite (Saari *et al*., 1990; Rajagopalan *et al*., 1996; Okamoto *et al*., 1997; 2001; Siwik *et al*., 2001). Another limitation that our study faced is the use of isolated, crystalloid-buffer-perfused hearts, where a role of basal NO may be overestimated. The perfusion flow ($10\,ml\,min^{-1}$), suggested by Langendorff (Doring & Dehnert, 1987) based on histological and metabolic factors, is higher than the physiologic *in vivo* coronary flow in the rats ($7–8\,ml\,min^{-1}\,g$ heart $weight^{-1}$) and this could cause an increase in the shear stress (Rubanyi *et al*., 1986), with a higher rate of basal NO biosynthesis in the coronary circulation. Moreover, the crystalloid solution used in our experimental conditions is very different from whole blood, and it is possible that the *in vitro* perfusion with Krebs–Henseleit solution can affect *per se* the release of MMP-2. However, the model of Langendorff perfused heart has always been considered a useful tool for the study of the coronary circulation and cardiac function, and we believe that it can give deep insight into the understanding of the local regulation of coronary parameters.

Taken together, our findings support the hypothesis that reducing endogenous NO biosynthesis by inhibition of NOS increases oxidative stress, which in turn leads to an increased MMP-2 activity in the coronary effluent. Our data, therefore, suggest a novel interaction between NO and MMP-2 in the coronary circulation.

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References

AMADO, J.A., SALAS, E., BOTANA, M.A., POVEDA, J.J. & BERRAZUETA, J.R. (1993). Low levels of intraplatelet cGMP in IDDM. *Diabetes Care,* **16,** 809–811.

BECKMAN, J.S., BECKMAN, T.W., CHEN, J., MARSHALL, P.A. & FREEMAN, B.A. (1990). Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc. Natl. Acad. Sci. U.S.A.,* **87,** 1620–1624.

CALVER, A., COLLIER, J., MONCADA, S. & VALLANCE, P. (1992). Effect of local intra-arterial N^G-monomethyl-L-arginine in patients with hypertension: the nitric oxide dilator mechanism appears abnormal. *J. Hypertens.,* **10,** 1025–1031.

CAO, M., STEFANOVIC-RACIC, M., GEORGESCU, H.I., MILLER, L.A. & EVANS, C.H. (1998). Generation of nitric oxide by lapine meniscal cells and its effect on matrix metabolism: stimulation of collagen production by arginine. *J. Orthop. Res.,* **16,** 104–111.

CHEN, H.H. & WANG, D.L. (2004). Nitric oxide inhibits matrix metalloproteinase-2 expression *via* the induction of activating transcription factor 3 in endothelial cells. *Mol. Pharmacol.,* **65,** 1130–1140.

CHEUNG, P.Y., SAWICKI, G., SALAS, E., ETCHES, P.C., SCHULZ, R. & RADOMSKI, M.W. (2000a). The mechanisms of platelet dysfunction during extracorporeal membrane oxygenation in critically ill neonates. *Crit. Care Med.,* **28,** 2584–2590.

CHEUNG, P.Y., SAWICKI, G., WOZNIAK, M., WANG, W., RADOMSKI, M.W. & SCHULZ, R. (2000b). Matrix metalloproteinase-2 contributes to ischemia–reperfusion injury in the heart. *Circulation,* **101,** 1833–1839.

CHEUNG, P.Y. & SCHULZ, R. (1997). Glutathione causes coronary vasodilation *via* a nitric oxide- and soluble guanylate cyclase-dependent mechanism. *Am. J. Physiol.,* **273,** H1231–H1238.

CLEUTJENS, J.P., KANDALA, J.C., GUARDA, E., GUNTAKA, R.V. & WEBER, K.T. (1995). Regulation of collagen degradation in the rat myocardium after infarction. *J. Mol. Cell. Cardiol.,* **27,** 1281–1292.

COKER, M.L., DOSCHER, M.A., THOMAS, C.V., GALIS, Z.S. & SPINALE, F.G. (1999). Matrix metalloproteinase synthesis and expression in isolated LV myocyte preparations. *Am. J. Physiol.,* **277,** H777–H787.

DE BELDER, A.J. & RADOMSKI, M.W. (1994). Nitric oxide in the clinical arena. *J. Hypertens.,* **12,** 617–624.

DONNINI, S., MORBIDELLI, L., TARABOLETTI, G. & ZICHE, M. (2004). ERK1-2 and p38 MAPK regulate MMP/TIMP balance and function in response to thrombospondin-1 fragments in the microvascular endothelium. *Life Sci.,* **74,** 2975–2985.

DORING, H.J. & DEHNERT, H. (1987). *The Isolated Perfused Heart According to Langendorff*. Buchenbach, Germany: BVM-Biomesstechnik.

GAVIN, P.J., CRAWFORD, S.E., SHULMAN, S.T., GARCIA, F.L. & ROWLEY, A.H. (2003). Systemic arterial expression of matrix metalloproteinases 2 and 9 in acute Kawasaki disease. *Arterioscler. Thromb. Vasc. Biol.,* **23,** 576–581.

GOSS, S.P., HOGG, N. & KALYANARAMAN, B. (1997). The effect of nitric oxide release rates on the oxidation of human low density lipoprotein. *J. Biol. Chem.,* **272,** 21647–21653.

JOHNSON III, G., TSAO, P.S. & LEFER, A.M. (1991). Cardioprotective effects of authentic nitric oxide in myocardial ischemia with reperfusion. *Crit. Care Med.,* **19,** 244–252.

JURASZ, P., SAWICKI, G., DUSZYK, M., SAWICKA, J., MIRANDA, C., MAYERS, I. & RADOMSKI, M.W. (2001). Matrix metalloproteinase 2 in tumor cell-induced platelet aggregation: regulation by nitric oxide. *Cancer Res.,* **61,** 376–382.

LUDMER, P.L., SELWYN, A.P., SHOOK, T.L., WAYNE, R.R., MUDGE, G.H., ALEXANDER, R.W. & GANZ, P. (1986). Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. *N. Engl. J. Med.,* **315,** 1046–1051.

MAINARDI, C.L., HIBBS, M.S., HASTY, K.A. & SEYER, J.M. (1984). Purification of a type V collagen degrading metalloproteinase from rabbit alveolar macrophages. *Coll. Relat. Res.,* **4,** 479–492.

MORIKAWA, E., HUANG, Z. & MOSKOWITZ, M.A. (1992). L-arginine decreases infarct size caused by middle cerebral arterial occlusion in SHR. *Am. J. Physiol.,* **263,** H1632–H1635.

MURPHY, G., REYNOLDS, J.J., BRETZ, U. & BAGGIOLINI, M. (1982). Partial purification of collagenase and gelatinase from human polymorphonuclear leucocytes. Analysis of their actions on soluble and insoluble collagens. *Biochem. J.,* **203,** 209–221.

NAGASE, H. (1997). Activation mechanisms of matrix metalloproteinases. *Biol. Chem.,* **378,** 151–160.

OKAMOTO, T., AKAIKE, T., NAGANO, T., MIYAJIMA, S., SUGA, M., ANDO, M., ICHIMORI, K. & MAEDA, H. (1997). Activation of human neutrophil procollagenase by nitrogen dioxide and peroxynitrite: a novel mechanism for procollagenase activation involving nitric oxide. *Arch. Biochem. Biophys.,* **342,** 261–274.

OKAMOTO, T., AKAIKE, T., SAWA, T., MIYAMOTO, Y., VAN DER VLIET, A. & MAEDA, H. (2001). Activation of matrix metalloproteinases by peroxynitrite-induced protein S-glutathiolation *via* disulfide S-oxide formation. *J. Biol. Chem.,* **276,** 29596–29602.

PAULUS, W.J. & SHAH, A.M. (1999). NO and cardiac diastolic function. *Cardiovasc. Res.,* **43,** 595–606.

RAJAGOPALAN, S., MENG, X.P., RAMASAMY, S., HARRISON, D.G. & GALIS, Z.S. (1996). Reactive oxygen species produced by macrophage-derived foam cells regulate the activity of vascular matrix metalloproteinases *in vitro*. Implications for atherosclerotic plaque stability. *J. Clin. Invest.,* **98,** 2572–2579.

RUBANYI, G.M., ROMERO, J.C. & VANHOUTTE, P.M. (1986). Flow-induced release of endothelium-derived relaxing factor. *Am. J. Physiol.,* **250,** H1145–H1149.

RUBBO, H., RADI, R., ANSELMI, D., KIRK, M., BARNES, S., BUTLER, J., EISERICH, J.P. & FREEMAN, B.A. (2000). Nitric oxide reaction with lipid peroxyl radicals spares alpha-tocopherol during lipid peroxidation. Greater oxidant protection from the pair nitric oxide/alpha-tocopherol than alpha-tocopherol/ascorbate. *J. Biol. Chem.,* **275,** 10812–10818.

RUBBO, H., RADI, R., TRUJILLO, M., TELLERI, R., KALYANARAMAN, B., BARNES, S., KIRK, M. & FREEMAN, B.A. (1994). Nitric oxide regulation of superoxide and peroxynitrite-dependent lipid peroxidation. Formation of novel nitrogen-containing oxidized lipid derivatives. *J. Biol. Chem.,* **269,** 26066–260675.

SAARI, H., SUOMALAINEN, K., LINDY, O., KONTTINEN, Y.T. & SORSA, T. (1990). Activation of latent human neutrophil collagenase by reactive oxygen species and serine proteases. *Biochem. Biophys. Res. Commun.,* **171,** 979–987.

SAWICKI, G., SALAS, E., MURAT, J., MISZTA-LANE, H. & RADOMSKI, M.W. (1997). Release of gelatinase A during platelet activation mediates aggregation. *Nature,* **386,** 616–619.

SIWIK, D.A., PAGANO, P.J. & COLUCCI, W.S. (2001). Oxidative stress regulates collagen synthesis and matrix metalloproteinase activity in cardiac fibroblasts. *Am. J. Physiol. Cell. Physiol.,* **280,** C53–C60.

SMITH, R.E., PALMER, R.M., BUCKNALL, C.A. & MONCADA, S. (1992). Role of nitric oxide synthesis in the regulation of coronary vascular tone in the isolated perfused rabbit heart. *Cardiovasc. Res.,* **26,** 508–512.

TROSTCHANSKY, A., BATTHYANY, C., BOTTI, H., RADI, R., DENICOLA, A. & RUBBO, H. (2001). Formation of lipid–protein adducts in low-density lipoprotein by fluxes of peroxynitrite and its inhibition by nitric oxide. *Arch. Biochem. Biophys.,* **395,** 225–232.

VALLANCE, P. & CHAN, N. (2001). Endothelial function and nitric oxide: clinical relevance. *Heart,* **85,** 342–350.

VAN WART, H.E. & BIRKEDAL-HANSEN, H. (1990). The cysteine switch: a principle of regulation of metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family. *Proc. Natl. Acad. Sci. U.S.A.,* **87,** 5578–5582.

WANG, W., SAWICKI, G. & SCHULZ, R. (2002a). Peroxynitrite-induced myocardial injury is mediated through matrix metalloproteinase-2. *Cardiovasc. Res.,* **53,** 165–174.

WANG, W., SCHULZE, C.J., SUAREZ-PINZON, W.L., DYCK, J.R., SAWICKI, G. & SCHULZ, R. (2002b). Intracellular action of matrix metalloproteinase-2 accounts for acute myocardial ischemia and reperfusion injury. *Circulation,* **106,** 1543–1549.

WINK, D.A., HANBAUER, I., KRISHNA, M.C., DEGRAFF, W., GAMSON, J. & MITCHELL, J.B. (1993). Nitric oxide protects against cellular damage and cytotoxicity from reactive oxygen species. *Proc. Natl. Acad. Sci. U.S.A.,* **90,** 9813–9817.

WOESSNER, J.F.J. (1998). The matrix metalloproteinases family. In: *Matrix Metalloproteinases,* ed. Parks, W.C. & Mecham, R.P. pp. 1–14. San Diego: Academic Press.

YASMIN, W., STRYNADKA, K.D. & SCHULZ, R. (1997). Generation of peroxynitrite contributes to ischemia–reperfusion injury in isolated rat hearts. *Cardiovasc. Res.,* **33,** 422–432.

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