British Journal of Pharmacology (1997) 120, 1128–1134

Nitric oxide modulation of transcellular biosynthesis of cys-leukotrienes in rabbit leukocyte-perfused heart

Carola Buccellati, Giuseppe Rossoni, Albino Bonazzi, *Ferruccio Berti, **Jacques Maclouf, [1]Giancarlo Folco & Angelo Sala

Center for Cardiopulmonary Pharmacology, Institute of Pharmacological Sciences, Via Balzaretti 9, 20133 Milan, Italy; *Department of Pharmacology, Chemotherapy and Toxicology, University of Milan, Italy and **U-348 INSERM, Hopital Lariboisière, 75475 Paris CEDEX 10, France

1 We have studied the role of nitric oxide (NO) in the regulation of the transcellular biosynthesis of sulphidopeptide leukotrienes (cys-LT) generated upon neutrophil-vascular wall interactions and their functional consequences, in the spontaneously beating, cell-perfused, heart of the rabbit.

2 Hearts were perfused under recirculating conditions (50 ml) with 5×10^6 purified human neutrophils (PMNL), and challenged with 0.5 μM A-23187 for 30 min. Coronary perfusion pressure (CPP) and left-ventricular end-diastolic pressure (LVEDP) were monitored. Cys-LT formation was measured by reversed phase high performance liquid chromatography (h.p.l.c.) and u.v. spectral analysis. Myeloperoxidase (MPO) enzyme activity, assayed in aliquots of the recirculating buffer, was used as a marker of PMNL adhesion to the coronary endothelium.

3 Basal CPP and LVEDP values averaged 45 ± 1.4 mmHg and 5 ± 0.1 mmHg, respectively; A-23187 triggered an increase in CPP (134 ± 9 mmHg, at 30 min) which was significantly attenuated by pretreatment with L-arginine, 100 μM (90 ± 3 mmHg, at 30 min). Pretreatment with N^G-monomethyl-L-arginine, 10 μM (L-NMMA), induced a marked increase in CPP (290 ± 40 mmHg, at 20 min) and in LVEDP (47 ± 16 mmHg), so pronounced that it caused cardiac arrest in systole in 5 out of 6 hearts and these were prevented by L-arginine, 100 μM (CPP 115 ± 10 mmHg, LVEDP 6 ± 1.1 mmHg, at 30 min).

4 The increase in CPP was accompanied by the release of cys-LT in the circulating buffer, which was reduced significantly by L-arginine. Pretreatment with L-NMMA, caused a marked rise in cys-LT concentrations which was prevented by L-arginine.

5 Neither L-arginine nor L-NMMA affected directly the A-23187-induced arachidonic acid (AA) metabolism in isolated PMNL alone.

6 Pretreatment with L-NMMA caused a prompt drop in myeloperoxidase (MPO) activity, suggesting rapid adhesion of PMNL to the coronary wall; this effect was significantly blunted by L-arginine.

7 This study suggests that NO provides cardioprotection in an organ model of transcellular metabolism of cys-LT by preventing PMNL adhesion to the coronary intima.

Keywords: Nitric oxide; sulphidopeptide leukotrienes; transcellular biosynthesis; polymorphonuclear leukocytes; L-arginine; N^G-monomethyl-L-arginine (L-NMMA); cardiac ischaemia; coronary circulation; cell-cell interactions

Introduction

Adhesion of polymorphonuclear leukocytes (PMNL) to vascular endothelial cells is a characteristic feature of inflammation (Grant, 1973); endothelial cells and neutrophils express adhesion proteins which mediate cell–cell interactions and regulate the transmigration of PMNL across the vascular endothelial barrier into the tissues, where they are involved in the immune response (Carlos & Harlan, 1994). Nitric oxide (NO), is a powerful, biologically active autacoid, which is formed by vascular endothelium and rapidly inactivated by superoxide (Gryglewski *et al*., 1986; Rubanyi & Vanhoutte, 1986). NO has been recently identified as a multifunctional mediator, produced by, and acting on, most cells of the body. Besides its function as a relaxing factor, a neurotransmitter and an immune defence molecule (Ignarro, 1990; Moncada *et al*., 1991), there is accumulating evidence that NO participates in the inhibition of platelet aggregation (Radomski *et al*., 1987a) and adhesion to endothelial monolayers (Radomski *et al*., 1987b) as well as in the prevention of PMNL activation (McCall *et al*., 1988). These latter properties of NO may be of relevance in ischaemia-reperfusion damage where PMNL play a key role in the propagation of reperfusion injury (Mullane, 1988) and where NO donors have been shown to exert a significant anti-adherence effect on PMNL (Lefer, 1992). The precise mechanism by which NO exerts these effects remains undefined. However, endogenous NO appears to play a significant role in inhibiting PMNL adherence to the endothelium in post-capillary venules, possibly via suppression of CD11/CD18 expression on PMNL (Kubes *et al*., 1991).

The microenvironment located at the interface between PMNL and vascular endothelial cells seems therefore to represent a strategic site which regulates the interplay between these cell populations. Pharmacological interventions which alter one or more factors involved in such cell–cell cross-talk, may have important repercussions in the control of vascular bed (e.g. in the coronaries) reactivity and function, representing a fundamentally important therapeutic target. Recently, the concept of inter-cellular cooperation, in the handling of unstable intermediates of eicosanoid biosynthesis (transcellular biosynthesis), has attracted a considerable interest. Original work of Marcus *et al*. (1980) has clearly shown that platelets cooperate with endothelial cells in the formation of prostacyclin (PGI_2) and similarly neutrophils interact with platelets as well as endothelial cells in generating sulphidopeptide leukotrienes (cys-LT) (Feinmark & Cannon, 1986; Maclouf & Murphy, 1988). The formation of cys-LT by cell–cell cooperation has been shown to cause a marked coronary vaso-

[1] Author for correspondence.

constriction accompanied by severe morphological inflammatory changes in the rabbit heart (Sala *et al*., 1993; 1996a).

In light of these findings, the importance of NO as a regulatory factor of cell-cell interaction, as well as of transcellular biosynthesis of cys-LT has been investigated in a model of PMNL-perfused rabbit heart. Moreover the capacity of nitric oxide to interfere with alterations of coronary tone and heart mechanics has been studied.

Methods

Hearts from male albino rabbits (BMG-Allevamento, Cividate al Piano, BG, Italy) weighing 2.5–2.8 kg, were isolated and perfused retrogradely through the aorta, as previously described (Berti *et al*., 1988; Sala *et al*., 1993), with Krebs Henseleit solution (mM: NaCl 137, KCl 4.0, $CaCl_2$ 2.0, NaH_2PO_4 1.8, $MgCl_2$ 1.8, $NaHCO_3$ 25 and glucose 5.5), areated with a gas mixture (95% O_2 + 5% CO_2) and kept at 37°C. Perfusion rate was maintained at 20 ml min^{-1} by a roller pump (GilsonMinipulse 2, Biolabo, Milan, Italy). A latex balloon was inserted into the left ventricular cavity for measurement of left ventricular end-diastolic pressure (LVEDP), recorded with a Hewlett Packard (Waltham, MA, U.S.A.) carrier amplifier (model 8805B) and recorder (model 7754A). The balloon was slowly filled with saline, to stabilize LVEDP at 5 mmHg. Hearts were equilibrated for 30 min and finally perfused in a recirculating system (50 ml); coronary perfusion pressure (CPP) and LVEDP were monitored continuously. Rabbit hearts were perfused with purified human neutrophils (PMNL), isolated from human blood (40 ml), withdrawn from healthy donors who had not received medications for at least 1 week and collected in 5.7 ml of ACD (citric acid monohydrate 41 mM; Na-citrate dihydrate 100 mM and glucose 136 mM). PMNL were obtained by dextran T-500 sedimentation (3% w/v in saline to a final 1.22%) of platelet rich plasma (PRP)-deprived human blood; erythrocytes were lysed and a Ficoll solution (Ficoll-Paque, Pharmacia) layered under the cell suspension and spun for PMNL purification (400 *g* for 30 min at room temperature).

PMNL (5×10^6 cells) suspension was supplied with Ca^{2+} (2 mM) and Mg^{2+} (0.5 mM) immediately before heart perfusion, diluted to 5 ml in Tyrode solution and added, at a flow rate of 0.6 ml min^{-1} to avoid mechanical obstruction, to 45 ml of the recirculating medium. A 15 min stabilization period, under recirculating conditions, was allowed before treatment with L-arginine (100 μM) or N^G-monomethyl-L-arginine (L-NMMA; 10 μM). When both L-arginine and L-NMMA were used, a 10 min interval was left between treatments; 10 min later, the PMNL suspension was added to the reservoir fluid. Challenge with A-23187 (0.5 μM) was performed 10 min after PMNL infusion and lasted for 30 min. At the end of the experiment, the entire heart reservoir fluid was withdrawn for storage under argon atmosphere at −20°C until high-performance liquid chromatography (h.p.l.c.) analysis.

Cell incubation

PMNL (5×10^6 ml^{-1} in 1 ml PBS) were supplied with Ca^{2+} (2 mM) and Mg^{2+} (0.5 mM) and incubated for 5 min at 37°C before addition of L-arginine (100 μM) and/or L-NMMA (10 μM); 10 min later, PMNL were challenged with A-23187, 0.5 μM, for 30 min. Stimulation was stopped by addition of ice-cold methanol (1 ml) and samples were stored at −20°C until analysis as previously described (Rossoni *et al*., 1996).

H.p.l.c. analysis

The entire heart reservoir fluid (approx. 45 ml) was collected, spiked with 50,000 d.p.m. [^{3}H]-LTD_4 as well as 25 ng of prostaglandin B_2 (PGB_2) and stored at −20°C until analysis. After centrifugation at 3,500 *g* for 15 min, 5 ml of phosphate buffer 1 M, pH 7.4 were added and the sample extracted by use of a solid phase cartridge (Mega Bond-Elut C8, Varian, Harbor City, CA, U.S.A.). The cartridge was washed with 5 ml of hexane and eluted first with 4 ml of ethylacetate : methanol (99 : 1) and then with 4 ml of methanol : water (90 : 10). The ethyl acetate fractions, containing PGB_2 and the dihydroxy-arachidonic acid derivative LTB_4, were dried, reconstituted in 0.6 ml of solvent A (methanol/acetonitrile/water/acetic acid, 10 : 10 : 80 : 0.02, v : v : v : v, pH 5.5 with ammonium hydroxide) and injected into an h.p.l.c. gradient pump system (Beckman mod. 126) connected to a diode array u.v. detector (Beckman mod. 168). U.v. absorbance was monitored at 280 nm, and full u.v. spectra (210–340 nm) acquired at a rate of 0.5 Hz. A multilinear gradient from solvent A to solvent B (50% methanol, 50% acetonitrile) was used. This method allows easy separation of LTB_4 from both 5S,12S-dihydroxy eicosatetraenoic acid (5S,12S-diHETE) and Δ6 trans LTB_4 epimers. The dried methanolic extracts, containing sulphidopeptide leukotrienes only, were reconstituted in h.p.l.c. solvent A (0.6 ml) containing 25 ng of PGB_2; radioactivity was measured with a β-counter (Packard mod. 4000, Milan, Italy) to assess recovery of cys-LT (40–70%). The remaining sample was injected into the same h.p.l.c. system used for LTB_4. The use of [^{3}H]-LTD_4 to monitor recovery was necessary because of the double extraction protocol used. Positive identification of cys-LT was obtained through u.v. spectral analysis of chromatographic peaks eluting at characteristic retention times. Quantitation was performed on positively identified peaks only, with standard curves of synthetic $LTC_4/D_4/E_4$ and LTB_4 (Cascade Biochem., Reading, UK) and values of cys-LT were corrected for recovery.

MPO assay

Myeloperoxidase (MPO) was used as a marker enzyme for measuring PMNL adhesion. Aliquots (1 ml) of the buffer recirculating through the isolated hearts were withdrawn at different time intervals and centrifuged at 12,000 *g* (2 min). The supernatant was carefully removed and cell pellets resuspended in 200 μl phosphate buffer, 50 mM, pH 6.0, containing 0.5%, w : v, hexamethyltetrammonium bromide (HTAB) and 0.1%, w : v, gelatine. Samples, kept on ice, were then sonicated in 4 cycles (15 s per cycle) and finally centrifuged at 13,000 *g* (15 min).

MPO activity was assayed in the supernatant by measuring the H_2O_2-dependent oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB) (Suzuki *et al*., 1983). The reaction was performed in a 96-wells microtitre plate; the reaction mixture consisted of 25 μl sample or MPO standard, 25 μl of a 16 mM solution of TMB in DMSO and 200 μl of 0.375 mM H_2O_2 in 0.08 M phosphate buffer, pH 5.4. This mixture was incubated for 5 min at 37°C and the reaction blocked by the addition of 25 μl of a 13.6 μg ml^{-1} solution of bovine catalase. In its oxidized form, TMB has a blue color, which was measured spectrophotometrically at 620 nm.

Drugs

Human MPO, L-arginine hydrochloride, L-NMMA, bovine catalase, HTAB, TMB were from Sigma-Aldrich (Milan, Italy). PGB_2, LTC_4, LTD_4, LTB_4 were from Cayman Chemical (Ann Arbor, MI, U.S.A.).

Statistics

In all experiments, differences between control and treated groups were analysed for statistical significance by a one-way analysis of variance (ANOVA) and Student's *t* test (two-tailed) for unpaired samples as appropriate. A value of $P < 0.05$ was accepted as significant.

In all tables and figures, results are expressed as mean ± s.e.mean.

Results

Effect on CPP

The rabbit isolated hearts, perfused under basal recirculating conditions at a constant flow of 20 ml min^{-1} for 30 min demonstrated an average CPP of 45 ± 1.4 mmHg; resistance to perfusion pressure did not change when unstimulated human PMNL (5×10^6 cells) were perfused through the rabbit isolated heart. However, challenge with the calcium ionophore A-23187 (0.5 μM) in the presence of PMNL, brought about a significant increase in CPP which started approximately 10 min after challenge and continued thereafter (Figure 1). Pre-challenge LVEDP values were 5 ± 0.1 mmHg and increased to 11.7 ± 6.7 mmHg after challenge with A-23187 in the presence of PMNL. Pretreatment (30 min before challenge) of the rabbit hearts with L-arginine (100 μM) did not affect coronary tone but resulted in a significant protection against the PMNL-dependent increase (Figure 1).

Pretreatment (20 min before challenge) of the rabbit hearts with L-NMMA (10 μM) caused a progressive increase in CPP which was slow in onset and unaffected by A-23187 (0.5 μM) (Figure 2). When L-NMMA-treated hearts were challenged in the presence of PMNL, a marked increase in CPP was seen, which was fast in onset and so pronounced as to cause cardiac arrest in systole in 5 out of 6 hearts at 20 min (Figure 2). LVEDP also increased markedly to 47 ± 16 mmHg ($P < 0.01$ vs control PMNL).

The striking increase in CPP observed in the presence of L-NMMA was fully prevented by L-arginine (Figure 3). L-Arginine pretreatment was also able to prevent the increase in LVEDP at 30 min after A-23187 challenge of recirculating PMNL; in fact, LVEDP values observed under these conditions were not different from pre-challenge values (6 ± 1 mmHg, $P < 0.01$ vs L-NMMA).

Leukotriene production of the PMNL-perfused isolated heart of rabbit

H.p.l.c. analysis of the final volume of the recirculating perfusate showed that, in the absence of PMNL, cys-LT (LTC_4-LTD_4) were almost undetectable (Figure 2). However, when challenge with A-23187 was carried out in the presence of PMNL, formation of cys-LT took place and allowed their positive identification by on-line u.v. spectrum analysis (Figure 1); following pretreatment with L-arginine, the amount of cys-LT detected in the perfusion buffer was significantly reduced (Figure 1).

Pretreatment of the rabbit hearts with L-NMMA, followed by challenge with A-23187 in the presence of PMNL, led to a two fold increase in cys-LT formation compared to untreated hearts (Figures 1 and 2), which was fully inhibited by L-arginine (Figure 3).

Cell incubation experiments

Challenge of purified human PMNL with A-23187 (0.5 μM for 30 min) resulted in the expected generation of LTB_4 as well as of its metabolites (20-hydroxy- and 20-carboxy-LTB_4), collectively referred to as LTBs. Trivial amounts of cys-LT were also detected, probably due to the presence of eosinophils in the PMNL preparation (Figure 4). Pretreatment of human PMNL with L-arginine, L-NMMA or their combination, did not interfere with the neutrophil metabolic pathway of arachidonic acid (AA).

Dynamics of recirculating PMNL in rabbit perfused heart

We evaluated myeloperoxidase enzyme activity (MPO) in aliquots (1 ml) of the recirculating buffer, taken at different time intervals, as an index of PMNL adhesion to the intima of the

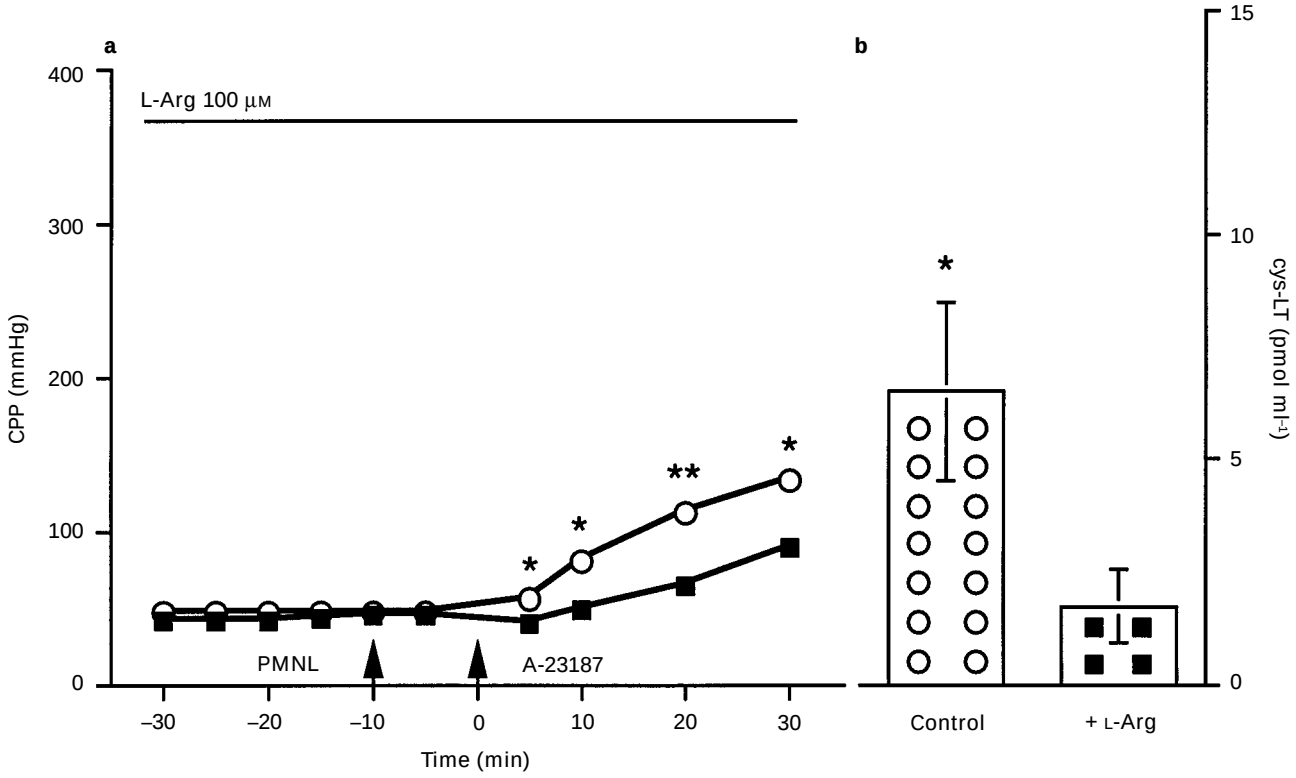

Figure 1 Effect of pretreatment (30 min) with L-arginine (■, 100 μM) on coronary perfusion pressure (CPP) (a) and levels of sulphidopeptide leukotrienes (cys-LT) (b) of rabbit isolated hearts perfused under recirculating conditions with human PMNL (1×10^5 cells ml^{-1}) and challenged with A-23187 (0.5 μM) (○, controls). Cys-LT were analysed by reverse-phase h.p.l.c. after extraction of whole buffer reservoir fluid, withdrawn 30 min after challenge. Values are mean ± s.e.mean ($n = 4$); s.e.mean are not shown when smaller than symbols. *$P < 0.05$; **$P < 0.01$ vs L-arginine (L-Arg).

coronary vascular bed. In samples withdrawn before PMNL addition, MPO was undetectable. However, in aliquots withdrawn immediately before A-23187 challenge, a significant presence of MPO could be detected, associated with the sample pellet. Pretreatment with L-NMMA (10 μM) caused a rapid disappearance of MPO activity from the recirculating buffer, suggesting intravascular adhesion of PMNL. When hearts were pretreated with L-arginine (100 μM) + L-NMMA, the

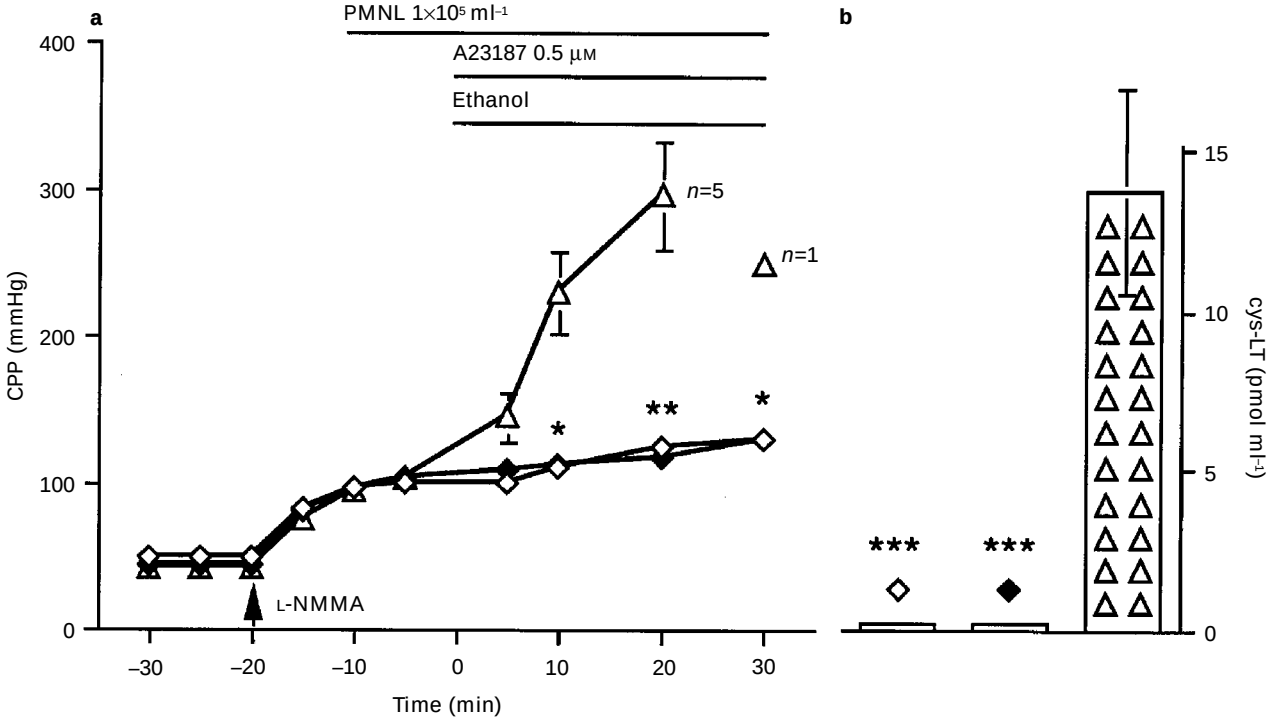

Figure 2 Effect of pretreatment (20 min) with L-NMMA (10 μM) on coronary perfusion pressure (CPP) (a) and levels of sulphidopeptide leukotrienes (cys-LT) (b) of rabbit isolated hearts perfused under recirculating conditions, with human PMNL (1×10^5 cells ml^{-1}) and challenged with A-23187 (0.5 μM) (△; $n=6$), or challenged with A-23187 in the absence of PMNL (◇; $n=3$), or challenged with vehicle (ethanol) (◆; $n=3$). Cys-LT were analysed by reverse-phase h.p.l.c. after extraction of whole buffer reservoir fluid, withdrawn 30 min after challenge. Values are mean ± s.e.mean; s.e.mean are not shown when smaller than symbols. $^*P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$ vs PMNL-perfused heart.

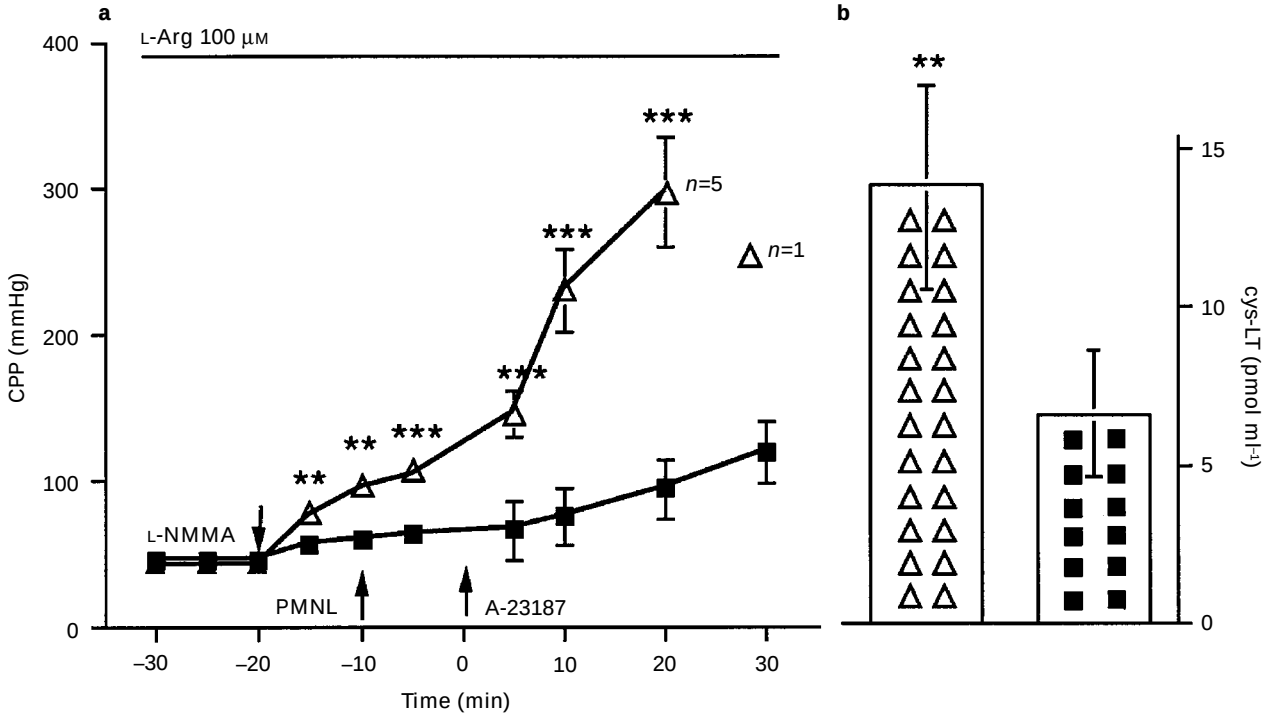

Figure 3 Prevention by L-arginine (L-Arg) treatment (■, 100 μM; $n=7$) of the effect of L-NMMA (△, 10 μM; $n=6$) on coronary perfusion pressure (CPP) (a) and levels of sulphidopeptide leukotrienes (cys-LT) (b) of rabbit isolated hearts perfused under recirculating conditions with human PMNL (1×10^5 cells ml^{-1}) and challenged with A-23187 (0.5 μM). Cys-LT were analysed by reverse-phase h.p.l.c. after extraction of whole buffer reservoir fluid, withdrawn 30 min after challenge. Values are mean ± s.e.mean; s.e.mean are not shown when smaller than symbols. $^{**}P<0.01$; $^{***}P<0.001$ vs L-Arg.

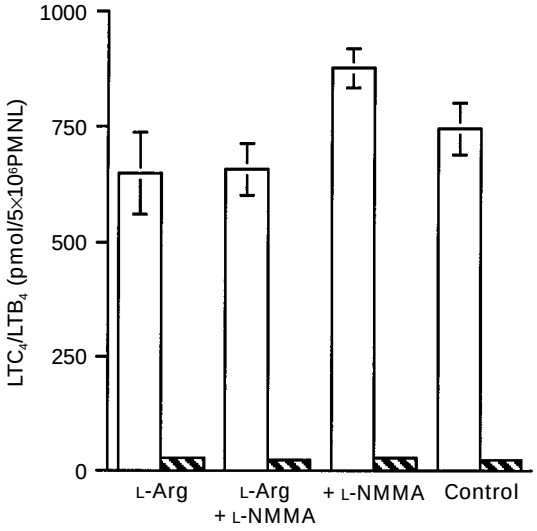

Figure 4 Effect of pretreatment with L-arginine (L-Arg, 100 μM, 30 min before challenge), L-NMMA (10 μM, 20 min) and their combination on the production of LTC_4 (hatched columns and LTB_4 (open columns) induced by challenge with A23187 (0.5 μM, 30 min) in human isolated purified PMNL (5×10^6 cells). Leukotrienes were analysed by reverse phase h.p.l.c. after solid-phase extraction. Values are mean ± s.e.mean, $n = 6$.

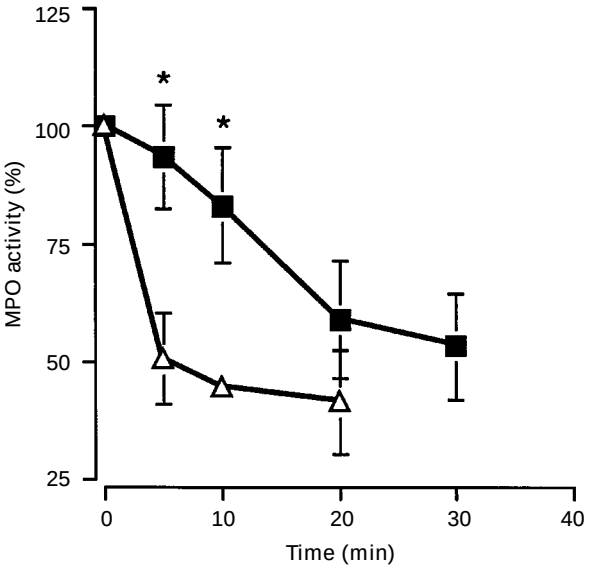

Figure 5 Effect of pretreatment with L-NMMA (△; 10 μM; $n = 3$) alone or in combination with L-arginine (■; 100 μM; $n = 4$) on myeloperoxidase (MPO) enzyme activity recovered from the recirculating buffer of rabbit isolated hearts perfused under recirculating conditions with human PMNL (1×10^5 cells ml^{-1}) and challenged with A-23187 (0.5 μM). $^*P < 0.05$ vs L-NMMA. Means are shown with vertical lines indicating s.e.mean.

drop of MPO activity was slower in onset and significantly less pronounced, indicating the persisting presence of PMNL in the recirculating buffer (Figure 5).

Moreover, none of the hearts ($n = 3$) treated with L-NMMA alone, survived till 30 min after challenge, whereas all the hearts ($n = 4$) treated with L-NMMA + L-arginine survived more than 30 min after A-23187 challenge.

Discussion

We have shown that pharmacological modulation of the NO pathway can markedly affect the cooperation between PMNL and coronary endothelial cells, in a model of rabbit PMNL-perfused heart, resulting in altered transcellular biosynthesis of sulphidopeptide leukotrienes and coronary tone. The present investigation follows previous studies in which we showed that challenge of PMNL, adhering to the coronary vascular wall, was accompanied by profound vasoconstriction and perivascular oedema, parallel to the formation of cys-LT, measured as LTC_4 and LTD_4 (Sala *et al.*, 1996a). The effects observed could not be reproduced by simple addition of high amounts (2 μg) of exogenous LTC_4, given as a bolus injection (personal unpublished observations), indicating the primary importance of local concentrations of cys-LT acting on target cells at the site of production, in good agreement with their autacoid nature. In this respect PMNL are cells which seem to be intended for transcellular biosynthesis of cys-LT, since a substantial proportion of their primary AA metabolites, i.e. the unstable epoxide leukotriene A_4 (LTA_4), is released outside the cells, where it becomes available to neighbouring acceptor cells (e.g. endothelial cells, EC) for further metabolism (Sala *et al.*, 1996b). This could lead to high local bioavailability of cys-LT which are known to constrict coronary arteries (Michelassi *et al.*, 1982) and have vasopermeant properties (Dahlen *et al.*, 1981) by contributing to endothelial cell retraction phenomena. This might have relevance for the extravasation of white cells from the vessel lumen to the tissue.

The interaction between PMNL and EC is controlled by a variety of factors that regulate the trafficking and migration of leukocytes. Both cells express on their surface pro-adhesive molecules, with diverse structures and mechanisms of expression, which may act as receptors to tether the two cells together or as signals that induce activation-dependent adhesion events (Zimmermann *et al.*, 1992). Less is known about locally produced factors that exert an inhibitory influence on leukoycte adhesion.

In recent years nitric oxide has attracted considerable interest as part of a widespread intercellular communication system. Two isoforms of NO synthase, the constitutive and the inducible enzyme, synthesize NO in the vascular system, by the stereospecific removal of the guanido nitrogen atom of L-arginine (White & Marletta, 1992). Endothelial cells possess the constitutive enzyme but are also able to express the inducible form by the action of bacterial products and/or cytokines (Rees *et al.*, 1990). Continuous generation of NO has been shown to play an important role in setting the resting tone of systemic as well as coronary resistance vessels (Huang *et al.*, 1995); in addition NO exerts several other effects including inhibition of PMNL activation and adherence (McCall *et al.*, 1989). These properties may be of considerable importance in explaining the modulatory role of NO on the transcellular biosynthesis of cys-LT, taking place between PMNL and coronary endothelial cells. Inhibition of NO synthesis resulted in an expected increase in coronary tone (Amezcua *et al.*, 1989), in a marked worsening of the PMNL-dependent and cys-LT-dependent coronary vasoconstriction as well as in enhanced biosynthesis of cys-LT. This was competitively antagonized by pretreatment with exogenous L-arginine, without direct action on either coronary perfusion pressure or 5-lipoxygenase enzyme activity. This indicates that the cardioprotective effect of NO is not simply restricted to the control of the coronary vascular tone, but may reside in its capacity to modulate the cross-talk between circulating cells and vessel wall. Increased adhesion of PMNL to the endothelium has been found following treatment with L-NMMA (Herbaczynska-Cedro *et al.*, 1991; Kubes *et al.*, 1994), and it is therefore plausible to hypothesize that under these conditions a facilitated transfer of LTA_4 might take place between donor cells (PMNL) and acceptor cells (coronary EC), where it becomes available for metabolism by the LTC_4-synthase.

The protection observed in the presence of L-arginine, is in line with previous findings, showing significant anti-adherence effects (Kubes *et al*., 1991; Lefer, 1992) and prevention of integrin-induced PMNL adhesion to postischaemic venules (Kubes *et al*., 1994).

The rapid disappearance of MPO enzyme activity that we observed in the circulating buffer following L-NMMA treatment, compared to its persistence in the presence of L-arginine, confirms the proposed role of nitric oxide as an endogenous modulator of PMNL adhesion. The exact mechanism linking inhibition of NO production with increased PMNL adherence is still undefined and may involve superoxide formation and mast cell activation (Kubes *et al*., 1993).

The biological role of NO in controlling vascular homeostasis may be potentially relevant in the pathogenesis of vascular diseases such as human coronary atherosclerosis, where the capacity of the endothelium to synthesize NO is reduced, or cardiac infarction and ischaemia-reperfusion injury, where PMNL are active participants in propagating tissue damage (Schmid-Schonbein, 1993) and increased urinary leukotriene excretion has been obtained (Carry *et al*., 1992). Research directed at the prophylactic use of agents which are able to prevent the adhesion of PMNL to the vascular endothelium and to cut off the biochemical interplay between these two cell populations, may prove effective in reducing the incidence of ischaemic heart disease and myocardial infarction.

Supported in part by the European Economic Community Concerted Action Contract N. BMHI CT93 1533.

References

AMEZCUA, J.L., PALMER, R.M.J., DESOUZA, B.M. & MONCADA, S. (1989). Nitric oxide synthesized from L-arginine regulates vascular tone in the coronary circulation of the rabbit. *Br. J. Pharmacol*., **97,** 1119 – 1124.

BERTI, F., ROSSONI, G., MAGNI, F., CARUSO, D., OMINI, C., PUGLISI, L. & GALLI, G. (1988). Non steroidal antiinflammatory drugs aggravate acute myocardial ischemia in perfused rabbit heart: a role for prostacyclin. *J. Cardiovasc. Pharmacol*., **12,** 438 – 444.

CARLOS, T.M. & HARLAN, J.M. (1994). Leukocyte-endothelial adhesion molecules. *Blood*, **84,** 2068 – 2101.

CARRY, M., KORLEY, V., WILLERSON, J.T., WEIGELT, L., FORD-HUTCHINSON, A.W. & TAGARI, P. (1992). Increased urinary leukotriene excretion in patients with cardiac ischemia: in vivo evidence for 5-lipoxygenase activation. *Circulation*, **85,** 230 – 236.

DAHLEN, S.E., BJORK, J., HEDQVIST, P., ARFORS, K.-E., HAMMARSTROM, S., LINDGREN, J.-A. & SAMUELSSON, B. (1981). Leukotrienes promote plasma leakage and leukocyte adhesion in postcapillary venules: *in vivo* effects with relevance to the acute inflammatory response. *Proc. Natl. Acad. Sci. U.S.A*., **78,** 3887 – 3891.

FEINMARK, S.J. & CANNON, P.J. (1986). Endothelial cell leukotriene C4 synthesis results from intracellular transfer of leukotriene A4 synthesized by polymorphonuclear leukocytes. *J. Biol. Chem*., **261,** 16466 – 16472.

GRANT, L. (1973). The sticking and emigration of white blood cells in inflammation. In *The Inflammatory Process*. ed. Grant, L., Zweifach, B.W. & McCluskey, R.T. pp. 205 – 249. New-York: Academic Press.

GRYGLEWSKI, R.J., PALMER, R.M.J. & MONCADA, S. (1986). Superoxide anion is involved in the breakdown of endothelium-derived relaxing factor. *Nature*, **320,** 454 – 456.

HERBACZYNSKA-CEDRO, K., LEMBOWICZ, K. & PYTEL, B. (1991). N^G-monomethyl-L-arginine increases platelet deposition on damaged endothelium *in vivo*. A scanning electron microscopy study. *Thromb. Res*., **64,** 1 – 9.

HUANG, P.L., HUANG, Z., MASHIMO, H., BLOCH, K.D., MOSKOWITZ, M.A., BEVAN, J.A. & FISHMAN, M.C. (1995). Hypertension in mice lacking the gene for endothelial nitric oxide synthase. *Nature*, **377,** 239 – 242.

IGNARRO, L.J. (1990). Biosynthesis and metabolism of endothelium-derived nitric oxide. *Ann. Rev. Pharmacol. Toxicol*., **30,** 535 – 560.

KUBES, P., KANWAR, S., NIU, X.F. & GABOURY, J.P. (1993). Nitric oxide synthesis inhibition induces leukocyte adhesion via superoxide and mast cells. *FASEB J*., **7,** 1293 – 1299.

KUBES, P., KUROSE, I. & GRANGER, D.N. (1994). NO donors prevent integrin-induced leukocyte adhesion but not P-selectin-dependent rolling in postischemic venules. *Am. J. Physiol*., **267,** H931 – H937.

KUBES, P., SUZUKI, M. & GRANGER, D.N. (1991). Nitric oxide: an endogenous modulator of leukocyte adhesion. *Proc. Natl. Acad. Sci. U.S.A*., **88,** 4651 – 4655.

LEFER, A.M. (1992). Cytoprotective actions of nitric oxide donors in ischemia-reperfusion injury. In *The Biology of Nitric Oxide. Enzymology, Biochemistry and Immunology*. ed. Moncada, S., Marletta, M.A., Hibbs, J.B. & Higgs, E.A. pp. 55 – 56. London: Portland Press.

MACLOUF, J. & MURPHY, R.C. (1988). Transcellular metabolism of neutrophil-derived leukotriene A4 by human platelets. *J. Biol. Chem*., **263,** 174 – 181.

MARCUS, A.J., WEKSLER, B.B., JAFFE, E.A. & BROEKMAN, M.J. (1980). Synthesis of prostacyclin from platelet-derived endoperoxides by cultured human endothelial cells. *J. Clin. Invest*., **66,** 979 – 987.

MCCALL, T., WHITTLE, B.J.R., BOUGHTON-SMITH, N.K. & MONCADA, S. (1988). Inhibition of FMLP-induced aggregation of rabbit neutrophils by NO. *Br. J. Pharmacol*., **95,** 517P.

MCCALL, T.B., BOUGHTON-SMITH, N.K., PALMER, R.M.J., WHITTLE, B.J.R. & MONCADA, S. (1989). Synthesis of nitric oxide from L-arginine by neutrophils. Release and interaction with superoxide ion. *Biochem. J*., **261,** 293 – 296.

MICHELASSI, F., LANDA, L., HILL, R.D., LOWENSTEIN, E., WATKINS, W.D., PETKAU, A.J. & ZAPOL, W.M. (1982). Leukotriene D4: a potent coronary artery vasoconstrictor associated with impaired ventricular contraction. *Science*, **217,** 841 – 843.

MONCADA, S., PALMER, R.M.J. & HIGGS, E.A. (1991). Nitric oxide: physiology, pathophysiology and pharmacology. *Pharmacol. Rev*., **43,** 109 – 142.

MULLANE, K.M. (1988). Myocardial ischemia-reperfusion injury: role of neutrophils and neutrophil derived mediators. In *Human Inflammatory Disease*. ed. Marone, G., Lichtenstein, L.M., Condorelli, M. & Fauci, A.S. Toronto; Philadelphia: B.C. Dekker Inc.

RADOMSKI, M.W., PALMER, R.M.J. & MONCADA, S. (1987a). Comparative pharmacology of endothelium-derived relaxing factor, nitric oxide and prostacyclin in platelets. *Br. J. Pharmacol*., **92,** 181 – 187.

RADOMSKI, M.W., PALMER, R.M.J. & MONCADA, S. (1987b). Endogenous nitric oxide inhibits human platelet adhesion to vascular endothelium. *Lancet*, **ii,** 1057 – 1058.

REES, D.D., CELLEK, S., PALMER, R.M.J. & MONCADA, S. (1990). Dexamethasone prevents the induction by endotoxin of a nitric oxide synthase and associated effects on vascular tone: an insight into endotoxin shock. *Biochem. Biophys. Res. Commun*., **173,** 541 – 547.

ROSSONI, G., SALA, A., BERTI, F., TESTA, T., BUCCELLATI, C., MOLTA, C., MULLER-PEDDINGHAUS, R., MACLOUF, J. & FOLCO, G.C. (1996). Myocardial protection by the leukotriene synthesis inhibitor BAY X1005: importance of transcellular biosynthesis of cysteinyl-leukotrienes. *J. Pharmacol. Exp. Ther*., **276,** 335 – 341.

RUBANYI, G.M. & VANHOUTTE, P.M. (1986). Superoxide anion and hyperoxia inactive endothelium-derived relaxing factor. *Am. J. Physiol*., **250,** H822 – 827.

SALA, A., ALIEV, G.M., ROSSONI, G., BERTI, F., BUCCELLATI, C., BURNSTOCK, G., FOLCO, G.C. & MACLOUF, J. (1996a). Morphological and functional changes of coronary vasculature caused by transcellular biosynthesis of sulfidopeptide leukotrienes in isolated heart of rabbit. *Blood*, **87,** 1824 – 1832.

SALA, A., BOLLA, M., ZARINI, S., MULLER-PEDDINGHAUS, R. & FOLCO, G.C. (1996b). Release of leukotriene A_4 versus leukotriene B_4 from human polymorphonuclear leukocytes. *J. Biol. Chem*., **271,** 17944 – 17948.

SALA, A., ROSSONI, G., BUCCELLATI, C., BERTI, F., FOLCO, G.C. & MACLOUF, J. (1993). Formation of sulphidopeptide-leukotrienes by cell-cell interaction causes coronary vasoconstriction in isolated, cell-perfused heart of rabbit. *Br. J. Pharmacol.*, **110,** 1206–1212.

SCHMID-SCHONBEIN, G.W. (1993). The damaging potential of leukocyte activation in the microcirculation. *Angiology*, **44,** 45–56.

SUZUKI, K., OTA, H., SASAGAWA, S., SAKATANI, T. & FUJIKURA, T. (1983). Assay method for myeloperoxidase in human polymorphonuclear leukocytes. *Anal. Biochem.*, **132,** 345–352.

WHITE, K.A. & MARLETTA, M.A. (1992). Nitric oxide synthase is a cytochrome P-450 type hemoprotein. *Biochemistry*, **31,** 6627–6631.

ZIMMERMAN, G.A., PRESCOTT, S.M. & MCINTYRE, T.M. (1992). Endothelial cell interactions with granulocytes: tethering and signaling molecules. *Immunology Today*, **13,** 93–100.

(Received July 31, 1996
Revised November 10, 1996
Accepted November 29, 1996)