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Inhibition by nitric oxide-releasing compounds of prostacyclin production in human endothelial cells

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1 The effects of two chemically unrelated nitric oxide (NO)-releasing compounds were studied on prostacyclin production in lipopolysaccharide (LPS)-stimulated human umbilical vein endothelial cells (HUVECs). The cells expressed cyclooxygenase-2 (COX-2) protein and produced prostacyclin by NS-398-sensitive manner suggesting that prostacyclin production derives principally by COX-2 pathway.

2 A novel NO-releasing oxatriazole derivative GEA 3175 (1–30 μM) inhibited LPS-induced production of prostacyclin in HUVECs in a dose-dependent manner being more potent than the earlier known NO-donor S-nitroso-*N*-acetylpenicillamine (SNAP).

3 The effects of the two NO-donors on prostacyclin synthesis were reversed when red blood cells were added into the culture indicating that the effects are due to NO released from the compounds.

4 Addition of exogenous arachidonic acid into the culture did not alter the inhibitory action of NO-donors suggesting that phospholipases are not the target of action of NO.

5 The NO-donors did not inhibit prostacyclin production in the presence of a selective COX-2 inhibitor NS-398. These data suggest that NO affects COX-2 pathway rather than has an overall effect on cyclooxygenases.

6 NO-releasing compounds did not alter the level of COX-2 protein expression in LPS-treated HUVECs as measured by Western blot analysis.

7 The results suggest that NO-donors inhibit the activity of COX-2 in human endothelial cells. A link between NO and the regulation of eicosanoid synthesis could represent an important mechanism in controlling vascular and inflammatory responses in pathophysiological states and during treatment with nitrovasodilators.

Keywords: Nitric oxide (NO); nitric oxide-releasing compounds; prostacyclin; cyclooxygenase (COX); endothelial cells

Introduction

Endothelial cells modulate vascular smooth muscle tone through release of vasoactive agents such as nitric oxide (NO), endothelin and eicosanoids. NO and prostacyclin are potent vasorelaxants and inhibitors of platelet aggregation that counterbalance the vasoconstrictor and platelet-aggregating properties of mediators like thromboxane and endothelin (Vane *et al*., 1990).

NO is derived from L-arginine by the enzyme nitric oxide synthase (NOS) whereas cyclooxygenase (COX) converts arachidonic acid to prostacyclin and other eicosanoids. There are two major forms of NOS and COX. The constitutively expressed isoforms of these enzymes are found in numerous cell types including endothelial cells. Production of NO and prostaglandins by the constitutive isoenzymes is implicated in the physiological regulation of vascular tone. The inducible isoforms (iNOS and COX-2) are not expressed in resting cells, but are induced following appropriate stimulation with pro-inflammatory agents such as lipopolysaccaride (LPS). (De-Witt, 1991; Knowles & Moncada, 1994).

NO interacts with heme-containing enzymes affecting their activation state (Henry *et al*., 1993). Binding of NO to the heme moiety of soluble guanylate cyclase results in an activation of this enzyme and enhanced production of cyclic GMP which mediates several cellular actions of NO (Ignarro, 1991). COX is a heme-containing enzyme catalyzing the formation of prostaglandins, prostacyclin and thromboxanes (Hla *et al*., 1993). The chemical interaction between NO and the heme moiety of COX has been demonstrated (Karthein *et al*., 1987). Depending on the cell type and experimental conditions used, NO has been found either to stimulate (Franchi *et al*., 1994; Davidge *et al*., 1995; Salvemini *et al*., 1996; Tetsuka *et al*., 1996), to inhibit (Stadler *et al*., 1993; Swierkosz *et al*., 1995; Minghetti *et al*., 1996; Amin *et al*., 1997) or not to influence (Tsai *et al*., 1994; Curtis *et al*., 1996; Järvinen *et al*., 1996) prostanoid synthesis. Furthermore, it has been shown that COX products negatively modulate the induction of the iNOS (Tetsuka *et al*., 1994). In spite of the active research in this field, the data from studies with human cells remains scarce.

NO is presently implicated as a key mediator of multitude of physiological processes and NO-donors have therapeutic potential in a range of pathologic conditions e.g. as vasodilators and anti-platelet drugs (Moncada & Higgs, 1995). Organic nitrates exert their pharmacological actions by releasing NO in enzymatic and nonenzymatic processes taking place mainly in vascular endothelium (Feelisch, 1993). Recently, groups of stucturally different molecules able to release NO spontaneously or after enzymatic conversion have been developed. The aim of the present study was to investigate the effects of nitrovasodilators on the synthesis of an endothelium-derived vasoactive and proinflammatory substance, prostacyclin, in *human* endothelial cells to predict

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indirect effects on endothelium in the regulation of vascular tone and inflammation. Two chemically unrelated NO-donors, GEA 3175 (Corell *et al*., 1994; Karup *et al*., 1994; Kankaanranta *et al*., 1996) and S-nitroso-*N*-acetylpenicillamine (SNAP; Feelisch, 1993) were used as representatives of NO-donors. The results suggest that NO-releasing compounds inhibit the activity of COX-2 enzyme in human endothelial cells.

Methods

Endothelial cell culture

Human umbilical vein endothelial cells (HUVECs) were isolated by treatment of human umbilical veins with 0.01% collagenase (Zimmerman *et al*., 1990). The cells were suspended in RPMI 1640 Glutamax-1 supplemented with 20% heat-inactivated fetal bovine serum, endothelial cell growth supplement (10 μg ml^{-1}), penicillin (100 units ml^{-1}), streptomycin (100 μg ml^{-1}) and amphotericin B (250 ng ml^{-1}). Cells were grown to confluence in plastic dishes, then removed by treatment with trypsin-EDTA (ethylenediamine tetraacetic acid), and seeded onto gelatin-coated 24-well plates. Red blood cells were isolated by Ficoll-Paque gradient centrifugation from human venous blood obtained from healthy volunteers who had abstained from any drugs for at least 1 week before sampling and suspended in culture medium described above.

Prostacyclin production

Confluent cultures of HUVECs were stimulated by lipopolysaccharide (LPS) for 6 h. NO-donors, red blood cells (RBC) and other compounds tested were added into the culture at the beginning of the incubations or at the time point indicated. The medium was collected and the stable metabolite of prostacyclin (6-keto-PGF$_{1\alpha}$) was measured by radioimmunoassay after Amprep C2 minicolumn purification.

Western blot analysis

Cell pellets from HUVECs were lysed in extraction buffer pH 7.4 at 4°C (10 mM Tris-base, 5 mM EDTA, 50 mM NaCl, 1% Triton-X-100, 0.5 mM phenylmethylsulfonyl fluoride, 2 mM Na-orthovanadate, 10 μg ml^{-1} leupeptin, 25 μg ml^{-1} aprotinin, 1.25 mM NaF, 1 mM Na-pyrophosphate, 10 mM n-octyl-β-D-glucopyranoside; all from Sigma, St. Louis, MO, U.S.A.). After centrifugation the supernatant was collected and an aliquot of the supernatant was used to determine protein by the Coomassie blue method (Bradford, 1976). Supernatants were then mixed 1 : 4 with sample buffer (62.5 mM Tris, pH 6.8, 10% glycerol, 2% SDS, 0.025% bromophenol blue and 5% β-mercaptoethanol; all from Sigma, St. Louis, MO, U.S.A.) and heated at 100°C for 5 min. Proteins were separated by SDS–PAGE on 10% polyacrylamide gels and then transferred to nitrocellulose. The non-specific binding was blocked with 5% non-fat milk, followed by incubation with goat polyclonal antibody (1 : 500 dilution) raised against a peptide corresponding to amino acids 27–46 mapping at the amino terminus of the COX-2 precursor (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.) for 45 min at room temperature. Membrane was then washed and incubated with a horseradish peroxidase linked anti-goat IgG (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.). The membrane was developed using the ECL™ Western blot detection system (Amersham International, Buckingshamshire, U.K.) and densitometric analysis was carried out using SigmaGel™ software.

Nitrite assay

Nitrite (NO_2^-), a stable product of NO in aqueous solutions was measured from the incubation medium with Griess reagent as previously described (Green *et al*., 1982). The detection limit of the assay was 1 μM (1 nmol/ 10^6 cells).

Drugs and chemicals

The mesoionic 3-aryl-substituted oxatriazole-5-imine derivative GEA 3175 as well as S-nitroso-*N*-acetylpenicillamine (SNAP) were kindly provided by GEA Ltd., Copenhagen, Denmark. Culture media, fetal bovine serum, antibiotics and trypsin-EDTA (Gibco, Paisley, Scotland, U.K.), collagenase A (Boehringer Mannheim, Germany), endothelial cell growth supplement, LPS, ketoprofen, cycloheximide and arachidonic acid (Sigma, St. Louis, MO, U.S.A), N-monomethyl-L-arginine (L-NMMA) (Clinalfa, Läufelfingen, Switzerland), L-N-iminoethyl-ornithine (L-NIO) and NS-398 (Alexis corp., Läufelfingen, Switzerland) were obtained as indicated. Radioimmunoassay kits for 6-keto-PGF$_{1\alpha}$ (a stable metabolite of prostacyclin) were from the Institute of Isotopes of Hungarian Academy of Sciences (Budapest, Hungary) and Amprep C2 minicolumns from Amersham International (Buckingshamshire, U.K.).

Results

Induction of prostacyclin production in HUVECs by LPS

LPS induced an accumulation of 6-keto-PGF$_{1\alpha}$ into the culture medium in a time- and dose-dependent manner (Figure 1). Cycloheximide (10 μg ml^{-1}) abolished the LPS-triggered increase in 6-keto-PGF$_{1\alpha}$ production (Figure 2) suggesting that LPS induced *de novo* synthesis of the enzyme COX-2 in HUVECs. A non-selective inhibitor of COX enzymes ketoprofen (30 μM) and a selective inhibitor of COX-2 NS-398 (1 μM) inhibited 6-keto-PGF$_{1\alpha}$ production both in the presence and absence of LPS by $>90\%$ (Figure 2) suggesting that most of the 6-keto-PGF$_{1\alpha}$ present in the culture medium was produced by COX-2. The induction of COX-2 synthesis by LPS was confirmed by Western blot analysis showing increased levels of COX-2 protein in LPS-treated cells. Cells incubated in the absence of LPS contained lower levels of COX-2 protein. Densitometric measurements showed that the band corresponding to COX-2 protein in control cells was $52 \pm 5\%$ ($n=7$) of the value in LPS (10 ng ml^{-1})-treated cells. This is consistent with the findings that 6-keto-PGF$_{1\alpha}$ production doubled from 1138 ± 235 pg ml^{-1} ($n=11$) to 2215 ± 192 pg ml^{-1} ($n=15$) when LPS (10 ng ml^{-1}) was added into the culture. These data indicate that HUVECs in the present cell culture conditions express COX-2 protein and produce prostacyclin predominantly by COX-2 when cultured in the presence and absence of LPS.

Effects of NO-donors on prostacyclin production in HUVECs

The two NO-donors tested inhibited LPS-induced production of 6-keto-PGF$_{1\alpha}$ in HUVECs in a dose-dependent manner. On

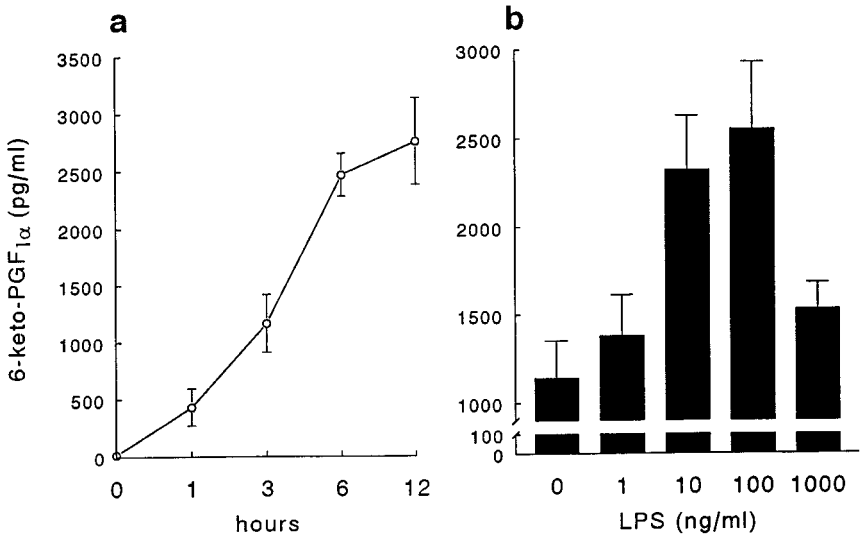

Figure 1 The effect of LPS on 6-keto-$PGF_{1\alpha}$ production in HUVECs. In (a) the cells were incubated with LPS (10 ng ml^{-1}) for the time indicated. In (b) the dose-response curve during a 6 h incubation time of LPS is shown. The stable metabolite of prostacyclin (6-keto-$PGF_{1\alpha}$) was measured by radioimmunoassay. The results are expressed as mean ± s.e.m. of four (a) or three (b) triplicate experiments.

molar basis the new oxatriazole derivative GEA 3175 was more potent than the earlier known NO-releasing compound S-nitroso-*N*-acetylpenicillamine (SNAP) (Figure 3). To study the possible effect of NO-donors on phospholipases, exogenous arachidonic acid was added into the culture. Arachidonic acid (30 μM) increased 6-keto-$PGF_{1\alpha}$ production up to 2.3-fold from 2644 ± 390 pg ml^{-1} ($n=4$) to 6204 ± 1850 pg ml^{-1} ($n=3$). The inhibition of prostacyclin synthesis by GEA 3175 was similar in the presence and absence of exogenous arachidonic acid (30 μM) (Figure 3a) suggesting that the actions of NO-donors are against cyclooxygenases rather than phospholipases. The addition of red blood cells (100 RBC/1EC) into the culture reversed the inhibitory action of NO-releasing compounds on 6-keto-$PGF_{1\alpha}$ synthesis in LPS-treated HUVECs indicating that the effect was due to NO released from the compounds (Figure 4).

To distinguish the effect of NO-donors on COX-2 from its possible overall effect on cyclooxygenases we tested the effects of NO-donors in the presence of a selective inhibitor of COX-2 NS-398. NS-398 (1 μM) inhibited LPS-induced 6-keto-$PGF_{1\alpha}$ production by 93% from 2184 ± 254 pg ml^{-1} to 151 ± 39 pg ml^{-1} ($n=5$). GEA 3175 and SNAP did not alter 6-keto-$PGF_{1\alpha}$ production in these culture conditions (Figure 5) suggesting that the enzyme COX-1 is not sensitive to the effect of NO-donors.

To obtain a more direct estimate of the action of NO-donors on cyclooxygenase activity in HUVECs, the cells were first stimulated with LPS (10 ng ml^{-1}) for 6 h to increase COX-2 expression, then washed and exposed to NO-donors for 30 min. The dose-dependent inhibitory action of GEA 3175 and SNAP on 6-keto-$PGF_{1\alpha}$ production was found also in these conditions (Figure 6) either in the presence or absence of exogenous arachidonic acid (30 μM). These results suggest that NO-releasing compounds inhibit the activity of COX-2 in LPS-treated HUVECs.

Effects of NO-donors on COX-2 protein levels in HUVECs

To determine whether the inhibition of 6-keto-$PGF_{1\alpha}$ production by NO-donors was due to a reduction of the amount of COX-2 protein, the Western blot analysis followed by densitometric measurements were carried out. As shown in Figure 7a a 71-kDa protein band corresponding to COX-2 was evident in the cell lysate from HUVECs. The levels of COX-2 protein were increased in response to 6 h incubation with LPS (10 ng ml^{-1}); densitometric measurements from control cells were 52 ± 5% ($n=7$) of values from LPS-stimulated cells. The levels of COX-2 protein in GEA 3175 and SNAP treated cells were 89 ± 3% ($n=4$) and 119 ± 13% ($n=3$) of that of LPS-

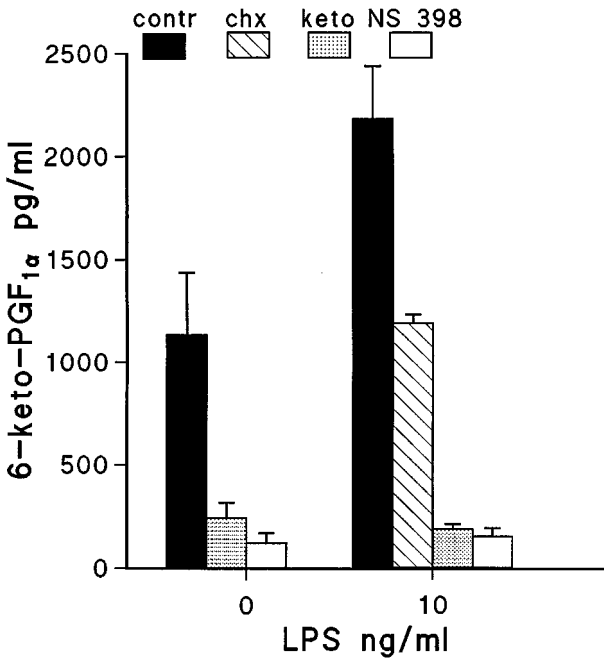

Figure 2 The effects of cycloheximide (chx; 10 μg ml^{-1}), ketoprofen (keto; 30 μM) and COX-2 inhibitor NS-398 (1 μM) on 6-keto-$PGF_{1\alpha}$ production in HUVECs. HUVECs were incubated with the compounds tested either in the presence or absence of LPS (10 ng ml^{-1}) for 6 h. The stable metabolite of prostacyclin (6-keto-$PGF_{1\alpha}$) was measured by radioimmunoassay. The results are expressed as mean ± s.e.m. of three to five duplicate experiments.

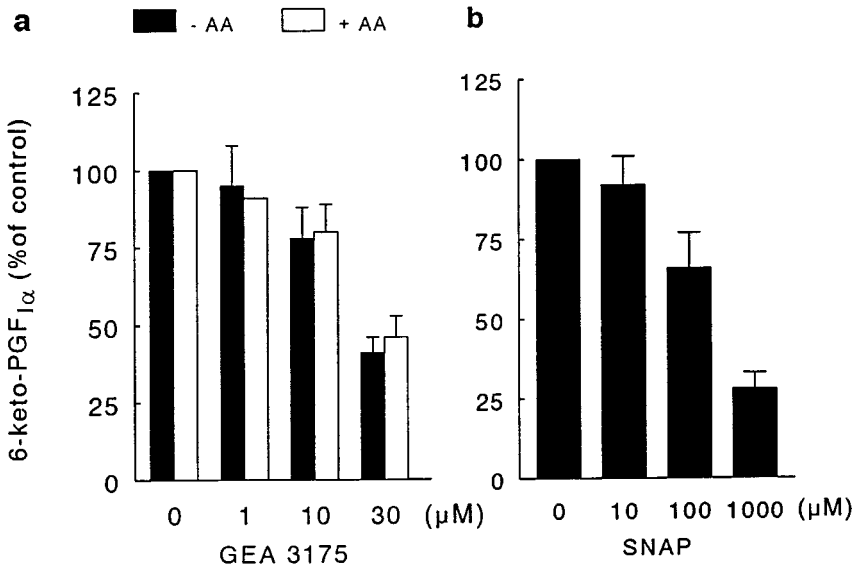

Figure 3 The effects of NO-donors on 6-keto-$PGF_{1\alpha}$ production in HUVECs. The stable metabolite of prostacyclin (6-keto-$PGF_{1\alpha}$) was measured by radioimmunoassay. The cells were incubated with GEA 3175 (a) or SNAP (b) and LPS (10 ng ml^{-1}) either in the presence (open columns) or absence (solid colums) of exogenous arachidonic acid (AA; 30 μM) for 6 h. The results are expressed as percentage of control (i.e. the cells cultured without NO-donor). The values are the mean$\pm$s.e.m. of three to four triplicate experiments. The actual values of 100% in (a) are 2644$\pm$390 pg ml^{-1} (without AA; $n=4$) and 6204$\pm$1850 pg ml^{-1} (with AA; $n=3$) and in (b) 2392$\pm$483 pg ml^{-1} ($n=4$).

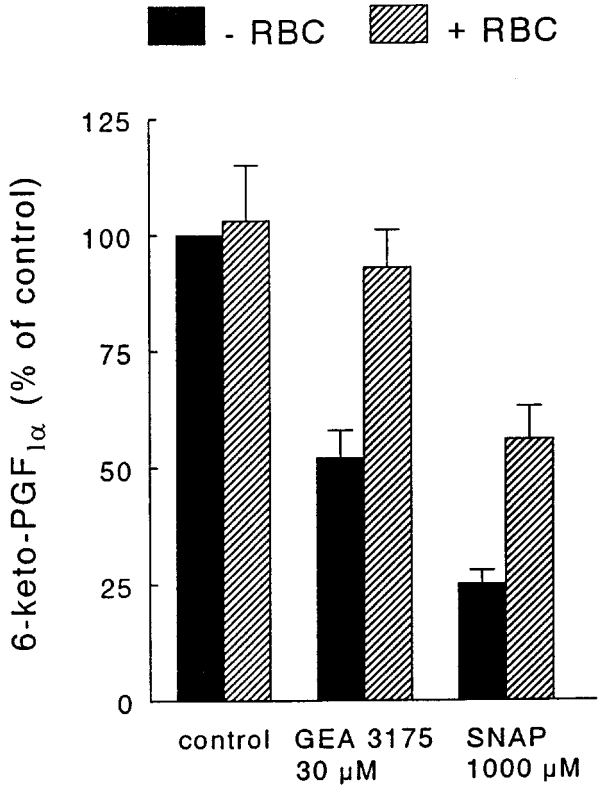

Figure 4 The effects of red blood cells on the action of NO-donors. The cells were cultured with LPS (10 ng ml^{-1}) and NO-donor for 6 h in the presence (hatched columns) or absence (solid columns) of red blood cells (100 RBC/1 EC). The stable metabolite of prostacyclin (6-keto-$PGF_{1\alpha}$) was measured by radioimmunoassay. The results are expressed as percentage of control (i.e. the cells cultured without NO-donor). The results are expressed as mean$\pm$s.e.m. of three to six triplicate experiments. The actual value of 100% is 2062$\pm$179 pg ml^{-1} ($n=6$).

stimulated controls, respectively (Figure 7b). These data suggest that NO-donors do not regulate prostanoid production at the level of COX-2 expression.

Effects of inhibitors of NOS on prostacyclin production in HUVECs

Inhibitors of NO synthase, L-NMMA (up to 1 mM) or L-NIO (up to 2 mM) did not alter LPS-induced 6-keto-$PGF_{1\alpha}$ production in HUVECs cultured in medium containing 950 μM L-arginine (Figure 8). When the experiments were repeated in the culture medium containing a more physiological concentrations of L-arginine (100 μM), 6-keto-$PGF_{1\alpha}$ production remained unchanged (data not shown; $n=4$). In addition, LPS (10 ng ml^{-1}) failed to induce detectable nitrite production in HUVECs (the detection limit was 1 μM).

Discussion

In the present study, we demonstrate that two chemically unrelated NO-releasing compounds suppress prostacyclin production in LPS-stimulated human endothelial cells. The results suggest that the activity but not the expression of COX-2 protein is inhibited.

GEA 3175 belongs to a family of recently characterized NO-releasing oxatriazole derivatives. The NO-releasing properties of these compounds have been characterized by their ability to inhibit platelet aggregation, induce cyclic GMP synthesis in platelets, convert oxyhemoglobin to methemoglobin, generate nitrite and nitrate in aqueous solutions and to form nitrosyl-hemoglobin complexes (Karup *et al.*, 1994; Kankaanranta *et al.*, 1996). The oxatriazole derivatives have been shown to have vasodilator, antiplatelet, fibrinolytic (Corell *et al.*, 1994) and antibacterial (Virta *et al.*, 1994) activities as well as to inhibit neutrophil functions (Moilanen *et al.*, 1993; 1994), suppress lymphocyte proliferation (Kosonen *et al.*, 1997), decrease tumour cell growth (Vilpo *et al.*, 1994),

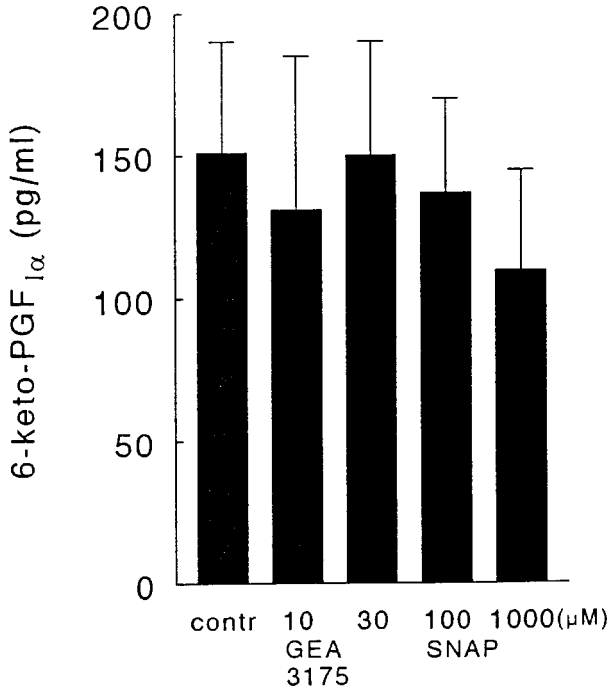

Figure 5 The effects of NO-donors on 6-keto-$PGF_{1\alpha}$ production in HUVECs in the presence of COX-2 inhibitor NS-398. The cells were incubated with NO-donors in the presence of LPS (10 ng ml^{-1}) and NS-398 (1 μM) for 6 h. The stable metabolite of prostacyclin (6-keto-$PGF_{1\alpha}$) was measured by radioimmunoassay. The values are the mean ± s.e.m. of four to five duplicate experiments.

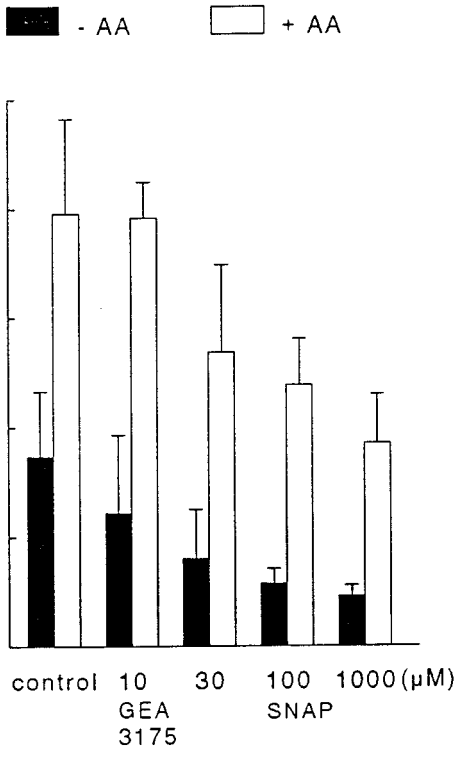

Figure 6 The effects of NO-donors on cyclooxygenase activity in HUVECs. The cells were first activated by LPS 10 ng ml^{-1} for 6 h, washed and then exposed to NO-donors for 30 min either in the presence (open columns) or absence (solid columns) of exogenous arachidonic acid (AA; 30 μM). The results are expressed as mean ± s.e.m. of three to four triplicate experiments.

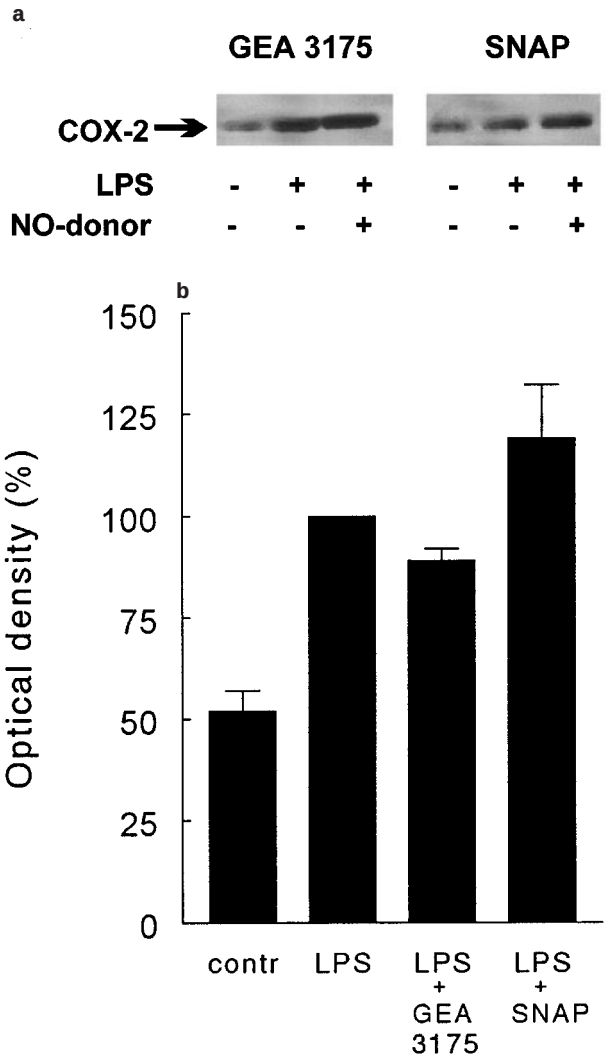

Figure 7 The effects of NO-donors on COX-2 protein levels in HUVECs. HUVECs were incubated for 6 h either in the presence or absence of LPS (10 ng ml^{-1}) and NO-donors GEA 3175 30 μM or SNAP 1000 μM. Cell lysates were separated by 10% SDS–PAGE, transferred to nitrocellulose membranes, and probed with a polyclonal antibody to COX-2. In (a) Western blot figures are representative of four (GEA 3175) or three (SNAP) separate experiments. In (b) densitometric measurements are shown. The results are expressed as percentage of LPS-stimulated cells (cultured without NO-donor). The values are the mean ± s.e.m. of three to seven experiments.

regulate glycosaminoglycan synthesis in articular cartilage (Järvinen *et al*., 1995) and inhibit oxidation of low density lipoprotein (Malo-Ranta *et al*., 1994).

On molar basis GEA 3175 was more potent than SNAP in its ability to inhibit prostacyclin synthesis in HUVECs. This agrees with our previous data on the order of potency of these NO-donors in their ability to increase cyclic GMP synthesis and augment other NO-like actions. The effects of the two NO-donors on prostacyclin synthesis were reversed by red cells added into the culture. Hemoglobin inhibits the action of NO by binding it to form nitrosylhemoglobin or by converting it to inactive nitrate (Henry *et al*., 1993; Murphy & Noack, 1994). Thus, these results suggest that the effects of GEA 3175 and SNAP are due to NO released from the compounds.

In the present experiments LPS-treated human endothelial cells were used to study the effects of NO-releasing compounds

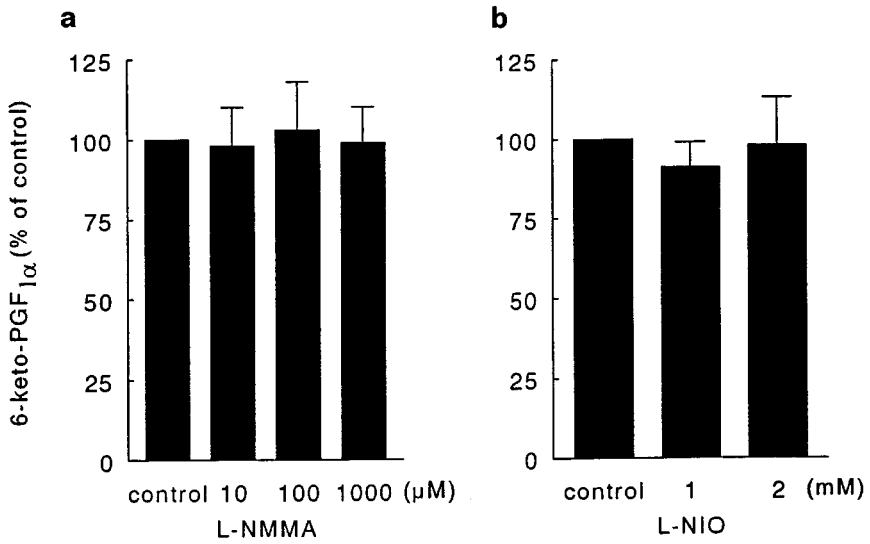

Figure 8 The effects of inhibitors of NO synthase L-NMMA and L-NIO on 6-keto-$PGF_{1\alpha}$ production in HUVECs. The cells were incubated with L-NMMA (a) or L-NIO (b) in the presence of LPS (10 ng ml^{-1}) for 6 h. The stable metabolite of prostacyclin (6-keto-$PGF_{1\alpha}$) was measured by radioimmunoassay. Incubation medium RPMI 1640 Glutamax-1 contained 950 μL L-arginine. The results are expressed as percentage of control (i.e. the cells cultured without NOS inhibitor). The values are the mean$\pm$s.e.m. of three to four triplicate experiments. The actual values of 100% are in (a) 2004$\pm$213 pg ml^{-1} ($n=4$) and in (b) 2392$\pm$483 pg ml^{-1} ($n=4$).

on prostacyclin formation. Two chemically unrelated NO-donors inhibited prostacyclin production (measured as 6-keto-$PGF_{1\alpha}$) in a dose-dependent manner. These experimental conditions measure prostacyclin production principally by COX-2 as evidenced by the effect of a spesific inhibitor of COX-2, NS-398 (Futaki *et al.*, 1994), which inhibited the accumulation of prostacyclin into the culture medium by $>90\%$. The presence of COX-2 protein in both unstimulated and LPS-stimulated cells was confirmed by Western blot analysis. This is consistent with the earlier data indicating that serum added into the culture medium contains factor(s) able to induce COX-2 in cell culture conditions (O'Banion *et al.*, 1992.). HUVECs unlike several other cells including bovine endothelial cells require serum and certain growth factors as supplements to culture medium to survive and replicate *in vitro*. The effect of NO-donors on COX-1 was tested in the presence of a selective inhibitor of COX-2 and no effect was found. In addition, phospholipases proved to be an unlike target of the inhibitory action of NO-donors because addition of exogenous arachidonic acid did not alter the response. These data suggest that NO-donors inhibit prostacyclin production in HUVECs by COX-2 pathway.

NO could regulate prostacyclin production by COX-2 at the level of enzyme activity or through the synthesis of the enzyme protein. In the present study, NO-donors inhibited prostacyclin production regardless of whether the compounds were added into the cultures together with LPS or 6 h afterwards at the time when a high-level induction of COX-2 was evident. These findings suggest that NO-donors inhibit the activity of COX-2 in cultured HUVECs. In further experiments NO-releasing compounds did not alter the amount of COX-2 protein in LPS-treated HUVECs implying that only the activity but not expression of COX-2 was affected.

Our results on the inhibitory action of NO on inducible eicosanoid synthesis are supported by findings of Stadler *et al.*, (1993); Swierkosz *et al.*, (1995) and Amin *et al.*, (1997). These articles report that NO inhibits prostanoid production by macrophages (Swierkosz *et al.*, 1995), Kupffer cells (Stadler *et al.*, 1993) or chondrocytes (Amin *et al.*, 1997) activated by inflammatory stimuli to express COX-2 activity. In contrast to these data, several studies in the literature indicate that NO enhances cyclooxygenase activity in various cell types including endothelial cells. Davidge *et al.* (1995) reported that endogenous NO increased eicosanoid production in bovine endothelial cells in response to calcium ionophore A23187 in culture conditions where Western blot analysis did not show any sign of induction of COX-2. In the study of Salvemini *et al.*, (1996) nitrovasodilators caused increased prostacyclin production in bovine aortic endothelial cells when incubated in arachidonic acid-supplemented Hank's balanced salt solution in the absence of serum and other factors known to induce COX-2. A direct NO-mediated activation of COX-1 has been shown after S-nitrosation of cysteine residues in the catalytic domain of the enzyme (Hajjar *et al.*, 1995). Based on these data it is tempting to speculate that the contradictory results on the effects of NO on eicosanoid synthesis could be explained not only by species- and tissue-specific responses or concentration-dependent biphasic activity of NO but may result from the different action of NO on COX-1 and COX-2 isoenzymes either directly or indirectly.

LPS induces expression of iNOS and production of high amounts of NO in several (mainly rodent) cell types whereas in human cells additional stimuli are usually needed (Nathan, 1992; Mossalayi *et al.*, 1994; Vouldoukis *et al.*, 1995). Presence of iNOS in human endothelial cells in tumour or inflammatory tissue has been described (Thomsen *et al.*, 1995; Sakurai *et al.*, 1995; Moilanen *et al.*, 1997) but the mechanism leading to induction of iNOS in this cell type remain to established. In the present study, no measurable nitrite production (detection limit 1 nmol 10^6 cells 6 h) as a marker of NO synthesis was found. Inhibitors of NOS failed to alter prostacyclin production in LPS-treated HUVECs. These results indicate that in LPS-treated HUVECs NO is produced in low (if any) concentrations and it does not regulate prostacyclin synthesis. This does not exclude the possibility that in the presence of additional factors (Rosenkranz-Weiss *et al.*, 1994) endogenous

NO might regulate COX-2 expression and/or activity in human endothelium *in vivo*.

In conclusion, the present results show that two chemically different NO-releasing compounds suppress prostacyclin production in LPS-treated *human* endothelial cells by inhibiting the activity but not the expression of COX-2. A link between NO and the regulation of eicosanoid synthesis could represent an important mechanism in controlling vascular and inflammatory responses in pathophysiological states and during treatment with nitrovasodilators.

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