# Cross-talk between group IIA-phospholipase $A_2$ and inducible NO-synthase in rat renal mesangial cells

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**1**   Features of glomerulonephritis are expression of the inducible form of NO synthase (iNOS) as well as expression of the secretory group IIA-phospholipase $A_2$ (sPLA$_2$) in mesangial cells. Interleukin 1$\beta$ (IL-1$\beta$) induces both enzymes with a similar time course resulting in an increase in nitrite production and sPLA$_2$-IIA activity. In this study we investigated the relationship between the formation of NO and sPLA$_2$-IIA induction in rat renal mesangial cells.

**2**   Incubation of mesangial cells with the NO-donor, spermine-NONOate, for 24 h induced sPLA$_2$-IIA mRNA expression and activity, whereas S-nitroso glutathione alone had only a small stimulatory effect. Stimulation of cells with IL-1$\beta$ caused a marked increase in sPLA$_2$-IIA mRNA and activity that were potentiated 3 fold by both NO donors.

**3**   Coincubation of cells with IL-1$\beta$ and the NOS inhibitor, L-N$^G$ monomethylarginine (L-NMMA), caused a dose-dependent inhibition of cytokine-induced sPLA$_2$-IIA mRNA expression and activity.

**4**   sPLA$_2$-IIA activity was not stimulated by 8-bromo-cyclic GMP indicating that NO-induced sPLA$_2$-IIA induction is independent of cyclic GMP-mediated signal transduction.

**5**   These data show that NO contributes to the expression by cytokines of sPLA$_2$-IIA and establishes a novel type of interaction between iNOS and sPLA$_2$-IIA in mesangial cells. This cross-talk between inflammatory mediators may help to promote and sustain an inflammatory state in the kidney.

**Keywords:**   cyclic GMP; phospholipase $A_2$; inducible NO synthase; mesangial cells; NO-donors; signal transduction

**Abbreviations:**   BSA, bovine serum albumine; cGMP, cyclic GMP; COX, cyclooxygenase; DMEM, Dulbecco's modified essential medium; GSNO, S-nitroso-glutathione; IL-1$\beta$, interleukin-1$\beta$; iNOS, inducible form of NO synthase; L-NMMA, L-N$^G$-monomethylarginine; NO, nitric oxide; spermine-NO, spermine-NONOate; sPLA$_2$-IIA, secretory phospholipase $A_2$-type IIA; TNF$\alpha$, tumour necrosis factor-$\alpha$

## Introduction

Group IIA phospholipase $A_2$ (sPLA$_2$-IIA) is an enzyme which is secreted from renal mesangial cells and several other cell types in response to proinflammatory cytokines such as interleukin-1$\beta$ (IL-1$\beta$) or tumour necrosis factor-$\alpha$ (TNF-$\alpha$) (for review see Pfeilschifter, 1995). The secreted enzyme is thought to participate in propagation of chronic inflammatory processes such as rheumatoid arthritis, sepsis and glomerulonephritis (for review see Vadas & Pruzanski, 1986; 1993). Moreover, it has been suggested that sPLA$_2$-IIA may act as an acute phase protein thereby enhancing inflammation and tissue damage (Hack et al., 1997; Pruzanski et al., 1998). The induction of sPLA$_2$-IIA mRNA and protein secretion occurs after cytokine-stimulation with a time course comparable to the gene expression of other proinflammatory enzymes. One such enzyme is the inducible nitric oxide synthase (iNOS). The product of iNOS, NO, has been implicated in a variety of fundamental biological pathways, including neurotransmission, vasodilatation and inflammation (for review, see Bredt & Snyder, 1994). NO exerts its effects on cell function through a variety of interactions, including binding to haem-containing moieties of enzymes such as soluble guanylate cyclase (Moncada & Higgs, 1993). The activation of guanylate cyclase and the resultant formation of cyclic GMP (cGMP) is responsible for several cellular signalling pathways.

Earlier studies have shown that, in rat mesangial cells, inflammatory cytokines such as IL-1$\beta$ or TNF$\alpha$ induce the expression of iNOS resulting in the production of large amounts of NO (Pfeilschifter & Schwarzenbach 1990; Pfeilschifter et al., 1992). Several proinflammatory effects of NO have been characterized in different cell types as model systems of glomerulonephritis such as generation of highly reactive peroxynitrite or hydroxyl radicals (Baud et al., 1992) or triggering of cell death by apoptosis or by necrosis (Mühl et al., 1996; Sandau et al., 1997). Evidence for the relevance of NO in inflammatory processes in the kidney has also been provided in animal models for certain types of glomerulonephritis (Cattell & Cook, 1993; 1995). The amplification of cytokine-stimulated iNOS expression by NO itself may be the basis for acute and chronic inflammatory processes in the kidney (Mühl & Pfeilschifter, 1995).

This effect of NO on gene transcription also indicates that NO may have the potential to regulate the expression of other inducible genes. In this respect in rat mesangial cells NO was found to be an important mediator of the regulation of cytokine-stimulated prostaglandin synthesis by activating cyclooxygenase-2 (COX-2; Tetsuka et al., 1996). The induction of prostaglandin biosynthesis is dependent on arachidonic acid release by the action of distinct phospholipases $A_2$ (Kramer & Sharp 1997; Hara et al., 1995; Murakami et al., 1998), and in particular the sPLA$_2$-IIA is reported to be important for initiation and propagation of prostaglandin $E_2$ formation in mesangial cells (Pfeilschifter et al., 1993).

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An interaction between NO and sPLA$_2$-IIA induction has not yet been addressed. In this study we evaluated the effects of NO on expression and activity of sPLA$_2$-IIA in IL-1$\beta$ stimulated rat mesangial cells. Our data highlight the existence of an efficient cross-communication between the NO- and eicosanoid-generating signalling pathways.

## Methods

### Cell culture

Rat renal mesangial cells were cultured as described previously (Pfeilschifter *et al*., 1984). The cells were grown in RPMI 1640 supplemented with 10% (v v$^{-1}$) foetal calf serum, penicillin (100 u ml$^{-1}$), streptomycin (100 $\mu$g ml$^{-1}$) and bovine insulin (0.66 u ml$^{-1}$). For experiments, cells were transferred to plastic Petri dishes (Greiner, Frickenhausen, Germany) with 3.5 cm or 10 cm diameter and were cultivated to near confluency. Twenty four hours prior to stimulation and during the experiments cells were starved by incubation in Dulbecco's modified essential medium (DMEM) containing 0.1 mg ml$^{-1}$ fatty acid-free bovine serum albumin (BSA) in the absence of serum.

### Nitrite analysis

Nitrite concentration was determined by the Griess reaction (Green *et al*., 1982). Cell culture supernatants were collected, and 200 $\mu$l were added into a 96-well plate (Greiner), and mixed with 20 $\mu$l sulphanilamide (dissolved in 1.2 M HCl) and 20 $\mu$l N-naphthylethylenediamine dihydrochloride. After 5 min at room temperature the absorbance was measured at 540 nm with an ELISA-reader (Biorad, München, Germany). Nitrite concentrations were calculated using sodium nitrite as standard.

### Phospholipase A$_2$ assay

Phospholipase A$_2$ activity in the supernatant of mesangial cell cultures was determined with [1-$^{14}$C]-oleate-labelled *Escherichia coli* as substrate as described previously (Märki & Franson, 1986). Assay mixtures (750 $\mu$l) contained Tris/HCl (pH 7.0) 100 mM, CaCl$_2$ 1 mM, [1-$^{14}$C]-oleate-labelled *E. coli* ($\approx$5000 c.p.m.) and the enzyme-containing supernatants of the cell cultures at a dilution producing 5% substrate hydrolysis. Reaction mixtures were incubated for 1 h at 37°C in a thermomixer. The reaction was stopped by the addition of 50 $\mu$l 1 mM EGTA/1 N HCl and 800 $\mu$l ethyl acetate. After extraction of the lipids the orgnaic phase was dried in a vacuum concentrator. Thereafter, the lipids were dissolved in 50 $\mu$l ethyl acetate and separated by thin layer chromatography on silicagel G 60 plates (Merck, Darmstadt) using the orgnaic phase of ethylacetate/isooctane/acetic acid/water (110/50/20/100 by vol.) as solvent system. The detection and quantification of the separated lipids was performed with a phosphorimager BAS 1500 from Fuji (Raytest, Straubenhardt, Germany). The sPLA$_2$-IIA-activity is defined as image quants counted from the spots corresponding to [1-$^{14}$C]-oleic acid. In parallel experiments extraction efficiency was determined to be greater than 95%.

### Northern-blot analysis

Confluent mesangial cells were cultured in 10 cm diameter culture dishes. After stimulation cells were washed twice with PBS and harvested using a rubber policeman. Total cellular RNA was extracted from the cell pellets using the guanidinium isothiocyanate/phenol/chloroform method (Sambrook *et al*., 1989). Samples of 10 $\mu$g of RNA were separated on 1.4% agarose/formaldehyde gels and transferred to a gene screen membrane. After UV crosslinking and prehybridization for 4 h the filters were hybridized for 16 h at 42°C to a $^{32}$P-labelled cDNA insert from sPLA$_2$-IIA (Van Schaik *et al*., 1993). DNA probes were radioactively labelled with $\alpha$-$^{32}$P-dCTP by random priming. Finally, the filters were washed twice with 2 $\times$ SSC/0.1% SDS for 2 $\times$ 20 min and several times at 65°C with 0.2 $\times$ SSC/1% SDS. The signal was detected and quantified with a phosphorimager BAS 1500 (see above). To correct for variations in RNA amount the sPLA$_2$-IIA-probe was stripped and the blots were rehybridized to the $\alpha$-$^{32}$P-dCTP-labelled cDNA insert for $\beta$-actin. The value of each of the RNA samples hybridized to the sPLA$_2$-IIA-probe was divided by that obtained by rehybridization of the blots to the $\beta$-actin probe.

### Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA). For multiple comparisons with the same control group, the limit of significance was divided by the number of comparisons according to Bonferroni.

### Materials

Recombinant IL-1$\beta$ was generously supplied by Dr C. Rordorf, Novartis Pharma Inc., Basel, Switzerland. [1-$^{14}$C]-oleic acid, and [$^{32}$P]-dCTP (110 TBq/mmol) were from Amersham-Buchler, Frankfurt, Germany. GSNO was kindly provided by Dr U.K. Messmer, Institute of Pharmacology, University of Frankfurt. Spermine-NONOate and L-N$^G$-monomethylarginine (L-NMMA) were obtained from Alexis (Grünberg, Germany). The cDNA clone coding for $\beta$-actin was a generous gift of Dr W. Eberhardt, Institute of Pharmacology, University of Frankfurt. Nylon membranes (Gene Screen) were purchased from NEN Life Science (Köln, Germany). All cell culture media and nutrients were from Gibco BRL (Eggenstein, Germany), and all other chemicals used were from either Merck (Darmstadt, Germany), Sigma (Munich, Germany) or Fluka (Deisenhofen, Germany).

## Results

### NO-donors GSNO and spermine-NO amplify IL-1$\beta$ stimulated sPLA$_2$-IIA induction

First we investigated whether the NO-donors S-nitrosoglutathione (GSNO) and spermine-NONOate (spermine-NO) have an influence on IL-1$\beta$ stimulated sPLA$_2$-IIA induction in mesangial cells. GSNO releases NO into the supernatant over a period of 2–3 h. Spermine-NO has a half life of about 45 min in cell culture and releases 2 moles of NO per mol spermine-NONOate, which leads to higher local concentrations of NO compared to GSNO (Feelisch, 1998; Maragos *et al*., 1991).

The results in Figure 1A show that a 24 h incubation with GSNO alone had no significant stimulatory effect on sPLA$_2$-IIA activity, whereas spermine-NO (Figure 1B) was able to increase the enzyme activity at 200 $\mu$M. After a 24 h coincubation with IL-1$\beta$ both compounds potentiated the sPLA$_2$-IIA activity about two to three times compared to IL-1$\beta$ alone. NO donors used up to concentrations of 200 $\mu$M had no cytotoxic effects during the time course of the experiments
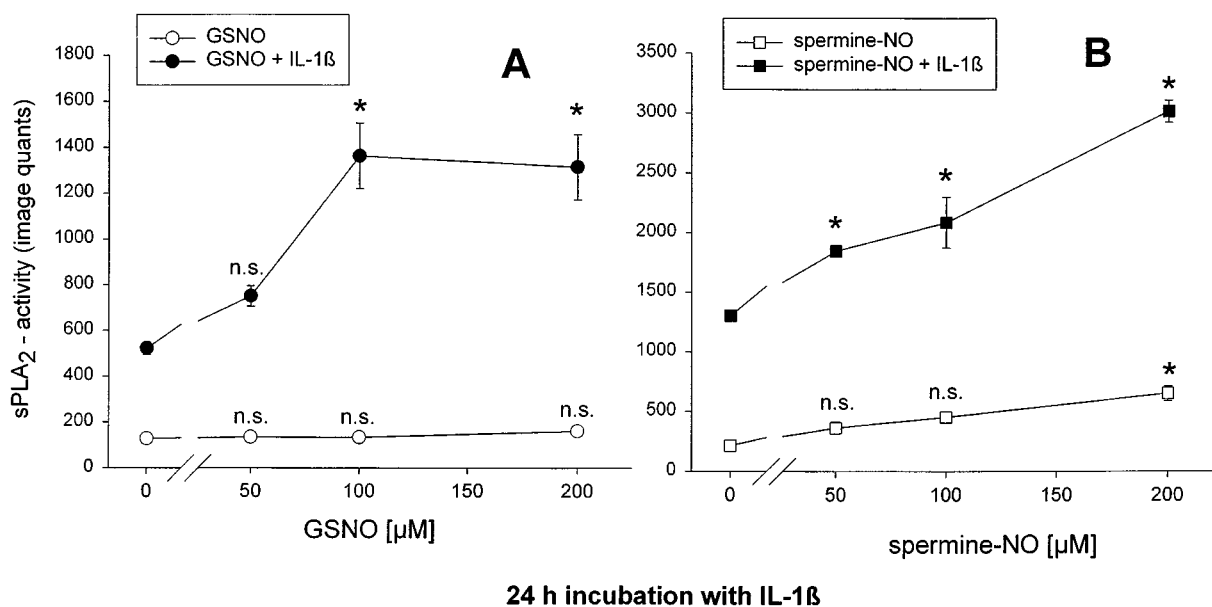
**Figure 1** Effect of the NO-donors GSNO (A) and spermine-NO (B) on IL-1$\beta$ stimulated sPLA$_2$-IIA activity in mesangial cells. Cells were incubated in serum-free DMEM with 0.1 mg ml$^{-1}$ fatty acid-free BSA for 24 h. Then they were treated for 24 h with IL-1$\beta$ (2 nM) or vehicle in the absence or presence of different concentrations of the NO-donors as indicated. sPLA$_2$-IIA activity was measured in the cell culture supernatant as described in Methods. Each value represents the means$\pm$s.e.mean of three parallel determinations per experiment. The experiment was repeated four times with similar results. Statistical analysis was performed by ANOVA. *$P<0.05$; n.s., not significant

as was monitored by trypan blue staining and lactate dehydrogenase release (data not shown).

By performing Northern blot analysis shown in Figure 2, lanes 1–6, we found that corresponding to the enzyme activity the increase in sPLA$_2$-IIA mRNA levels in the presence of spermine-NO alone was more pronounced than in the case of GSNO alone. However, in combination with IL-1$\beta$, both NO-donors potentiated the amount of sPLA$_2$-IIA mRNA up to 2.7$\pm$0.8 fold ($n=5$), when compared to IL-1$\beta$ alone ($P<0.05$; Student's $t$-test).

### L-NMMA inhibits nitrite formation and sPLA$_2$-IIA induction

In a second approach cells were treated with IL-1$\beta$ in the presence of L-NMMA, an inhibitor of iNOS activity.

The data in Figure 3 show that with 4 mM L-NMMA, a concentration at which IL-1$\beta$ stimulated nitrite formation was completely abolished (Figure 3A), the sPLA$_2$-IIA activity was reduced to about 15% of the IL-1$\beta$ induced activity (Figure 3B). At 1 mM L-NMMA, which reduced the concentration of nitrite in the cell culture medium by 90%, an inhibition of sPLA$_2$-IIA activity of about 50% was obtained.

Under these conditions we performed Northern blot analysis (Figure 2, lanes 7–10). Similar to its effect on the enzyme activity L-NMMA dose-dependently reduced the sPLA$_2$-IIA mRNA levels by 50% at 1 mM (lane 8) and 86% of IL-1$\beta$ at 4 mM (lane 10). In four separate experiment with L-NMMA-concentrations between 3 and 5 mM the reduction of IL-1$\beta$-induced mRNA levels varied between 53 and 86%. L-NMMA alone had no effect on basal sPLA$_2$-IIA-mRNA amounts (lanes 7 and 9). The results show that under conditions of complete inhibition of nitrite formation, the induction of sPLA$_2$-IIA mRNA is also reduced, although not to the same magnitude.
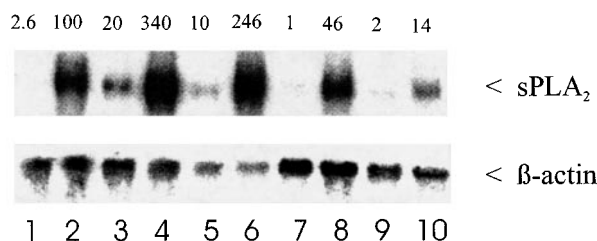


**Figure 2** Effect of GSNO, spermine-NO and L-NMMA on sPLA$_2$-IIA mRNA expression in mesangial cells. Cells were treated for 24 h with IL-1$\beta$ (lane 2) or vehicle (lane 1) in absence or presence of the NO-donors or of different concentrations of L-NMMA. Total cellular RNA was successively hybridized to $^{32}$P-labelled sPLA$_2$-IIA and $\beta$-actin cDNA probes, and quantification was performed as described in Methods. The numbers at the top of the Northern blot represent the corrected density expressed as the percentage of RNA found in IL-1$\beta$ stimulated cells. This is a representative Northern blot out of five separate experiments. Lane 1, control; lane 2, IL-1$\beta$ (2 nM); lane 3, spermine-NO (200 $\mu$M); lane 4, spermine-NO (200 $\mu$M) plus IL-1$\beta$; lane 5, GSNO (200 $\mu$M); lane 6, GSNO (200 $\mu$M) plus IL-1$\beta$; lane 7, L-NMMA (1 mM); lane 8, L-NMMA (1 mM) plus IL-1$\beta$; lane 9, L-NMMA (4 mM); lane 10, L-NMMA (4 mM) plus IL-1$\beta$

### sPLA$_2$-IIA induction is not changed by 8-bromo-cyclic GMP

cyclic GMP represents an important second messenger in NO-mediated signalling. Therefore we investigated whether the cell-permeable 8-bromo-cyclic GMP analogue has an effect on IL-1$\beta$ induced sPLA$_2$-IIA activity. Incubation of mesangial cells for 24 h with 8-bromo-cyclic GMP (500 $\mu$M) had no stimulatory effects on either nitrite formation or on sPLA$_2$-IIA activity (Table 1). A 24 h coincubation of mesangial cells with 8-bromo-cyclic GMP and IL-1$\beta$ did not result in a potentiation
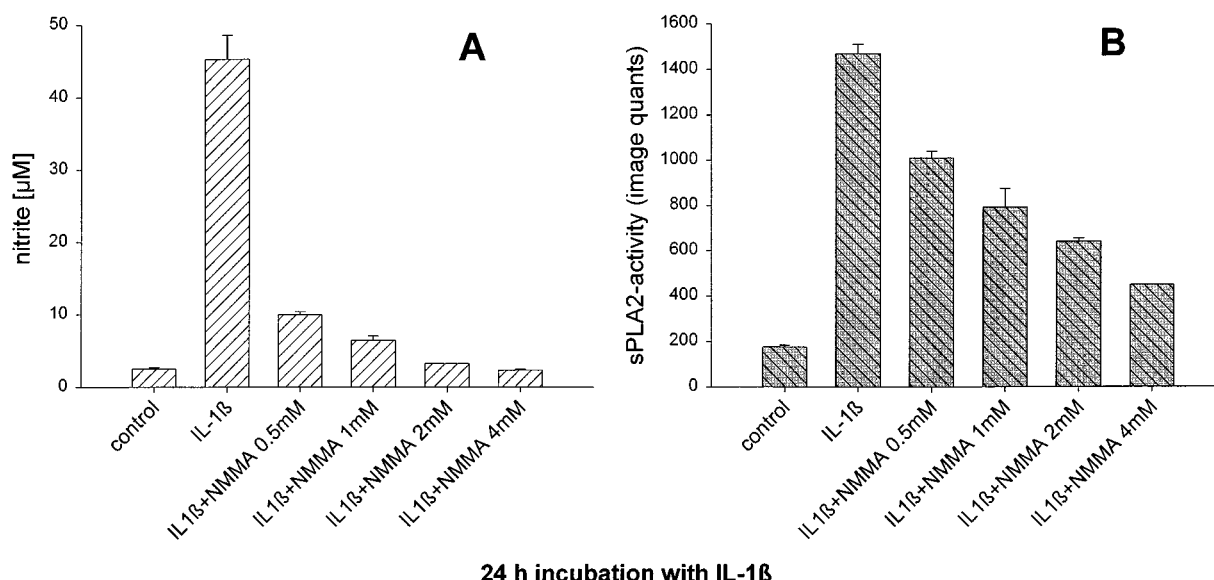
**Figure 3** Effect of L-NMMA on IL-1$\beta$ stimulated nitrite formation (A) and sPLA$_2$-IIA activity (B) in mesangial cells. Cells cultivated for 24 h in serum-free DMEM with 0.1 mg ml$^{-1}$ BSA were treated for 24 h with IL-1$\beta$ (2 nM) or vehicle in the absence or presence of different concentrations of L-NMMA as indicated. The concentration of nitrite as well as sPLA$_2$-IIA activity in the cell culture medium were determined as described in Methods. Each value represents the means$\pm$s.e.mean of three parallel determinations per experiment. The experiment was repeated four times with similar results.

**Table 1** Effect of 8-bromo-cGMP on nitrite formation and sPLA$_2$-IIA activity

| Treatment | Nitrite ($\mu$M) | sPLA$_2$-IIA activity (image quants) |
|---|---|---|
| Control | 2.5 $\pm$ 0.18 | 175 $\pm$ 9 |
| 8-bromo-cGMP | 1.8 $\pm$ 0.07 | 167 $\pm$ 10 |
| IL-1$\beta$ | 45.2 $\pm$ 3.4 | 1470 $\pm$ 39 |
| IL-1$\beta$ + 8-bromo-cGMP | 41.7 $\pm$ 3.8 | 1377 $\pm$ 29 |

Cells were treated for 24 h with 8-bromo-cGMP (500 $\mu$M) in absence or presence of IL-1$\beta$ (2 nM) as indicated. sPLA$_2$-IIA activity and nitrite were measured in the cell culture supernatant as described in Methods. Each value represents the mean of three determinations per experiment$\pm$s.e. The experiment was repeated three times with similar results.

of sPLA$_2$-IIA activity, suggesting that the effect of NO on sPLA$_2$-IIA is independent of cGMP-mediated signalling pathways.

## Discussion

Mesangial cells have been shown to express iNOS and sPLA$_2$-IIA in response to inflammatory cytokines such as IL-1$\beta$ (for review, see Pfeilschifter, 1994; 1995). A novel finding in our studies is that endogenous NO produced after cytokine stimulation of mesangial cells contributes to the induction of gene expression and activity of sPLA$_2$-IIA. The participation of iNOS activation and NO in sPLA$_2$-IIA gene expression was shown by the results obtained with L-NMMA plus IL-1$\beta$ where a complete inhibition of nitrite formation resulted in a substantial decrease in sPLA$_2$-IIA. However, the reduction of sPLA$_2$-IIA did not reach basal levels, suggesting that NO is important but not by itself sufficient for sPLA$_2$-IIA gene expression. The cytokine-treatment is necessary for this event and seems to initiate additional NO-independent mechanisms for sPLA$_2$-IIA induction.

Further support for a regulatory role of NO on sPLA$_2$-IIA gene expression was obtained by the use of different NO-donors. NO derived from spermine-NO as well as GSNO stimulated sPLA$_2$-IIA mRNA expression and activity in combination with IL-1$\beta$, whereas, when given alone only spermine-NO had a stimulatory effect. The reasons for the discrepancy in the effects on sPLA$_2$-IIA gene expression probably lie in the different capacities of these compound classes to release NO (for review see Feelisch, 1998). The release of 2 moles of NO from spermine-NO occurs with a much faster dissociation rate than the release of 1 mole from GSNO. This likely results in a higher local concentration of NO after spermine-NO-treatment of cells, and it is possible that a certain threshold must be reached in order to achieve induction of sPLA$_2$-IIA gene expression. Thus, the same absolute amounts of NO generated over different periods of time may lead to substantially different rates of NO formation and, consequently, to different effects on cellular responses.

The regulation of gene expression by NO impacts on a variety of proinflammatory mediators. In mesangial cells NO functions in a positive feedback loop that strengthens its own biosynthesis by potentiating iNOS expression (Mühl & Pfeilschifter, 1995). Endogenous NO as well as NO-donors have been implicated in the production of IL-6 and IL-8 in LPS-stimulated whole blood cells, fibroblasts, epithelial cells and hepatoma cells (Remick & Villarete, 1996; Villarete & Remick, 1997) and in a melanoma cell line (Andrew *et al.*, 1995) by affecting regulation at the transcriptional level.

Endogenously formed NO is a potent inducer of COX-2 activity (Salvemini *et al.*, 1993; Swierkosz *et al.*, 1995). NO is hypothesized to interact with the iron-haem centre of the active site of the COX enzymes thereby modulating enzyme activity (Salvemini *et al.*, 1993). In addition, NO stimulates prostaglandin formation in animal models of inflammation (Salvemini *et al.*, 1994; Salvemini, 1997; Sautebin *et al.*, 1995) and in several cell systems such as airway epithelial cells (Watkins *et al.*, 1997), osteoblasts (Kanematsu *et al.*, 1997) and astroglial cells (Mollace *et al.*, 1998). Furthermore, in

mesangial cells, NO donors not only stimulate COX-2 activity, but are also potent amplifiers of IL-1$\beta$-induced COX-2 mRNA and protein expression (Tetsuka *et al.*, 1996). However, inhibitory effects of NO donors on COX-2 activity and induction, when applied in higher concentrations, have also been described (Swierkosz *et al.*, 1995). Taken together, this evidence indicates that a fine-tuning of NO production seems to be crucial for the ultimate stimulation or inhibition of COX-2 induction.

Prostaglandins as products of COX activity play a major role as mediators of the inflammatory response. The rate-limiting step for prostaglandin biosynthesis is the availability of arachidonic acid as substrate for the COX enzymes. The most important route for arachidonic acid release from phospholipids appears to be by the action of a cytosolic PLA$_2$ (Kramer & Sharp, 1997). However, in mesangial cells the extracellular sPLA$_2$-IIA has been shown to be crucial for potentiation of cPLA$_2$ activity *via* activation of the c-Raf/MAP-kinase cascade and for sustained prostaglandin E$_2$ formation. This effect was described as cross-talk between sPLA$_2$-IIA and cPLA$_2$ by Huwiler *et al.* (1997). Here we have shown for the first time that in mesangial cells NO regulated gene expression involves also sPLA$_2$-IIA as an important factor upstream of COX-2 induction and prostaglandin formation.

The mechanisms by which NO modulates sPLA$_2$-IIA gene expression are unknown. The question arises whether the effect of NO on sPLA$_2$-IIA induction is mediated by activation of the soluble guanylate cyclase producing cyclic GMP. We found that the effects of IL-1$\beta$ on iNOS as well as sPLA$_2$-IIA do not seem to be mediated by this second messenger, because the cell-permeable analogue 8-bromo-cyclic GMP alone neither activated these enzymes nor potentiated the IL-1$\beta$ induced effects. These results are in agreement with observations reported by Mühl & Pfeilschifter (1995), where dibutyryl-cyclic GMP did also not mimic the amplification of iNOS expression in response to NO donors. Other groups have also found that NO-mediated iNOS- and COX-2-expression is regulated by cyclic GMP-independent mechanisms (Salvemini *et al.*, 1993; Sautebin *et al.*, 1995). In contrast, a cyclic GMP-dependent prostaglandin E$_2$ formation in mesangial cells was described by Tetsuka *et al.* (1996) and in airway epithelial cells by Watkins *et al.* (1997). This diversity in the cellular mechanisms might be due to cell- and tissue-specific differences in targets for this second messenger such as the cyclic GMP-dependent protein kinase.

Our results show that in mesangial cells NO has a role as an upstream mediator in the cytokine-induced signalling cascade, which leads to sPLA$_2$-IIA induction, whereby prostaglandin formation might be potentiated. In other words, an inhibition of NO production also is likely to reduce formation of other potent proinflammatory mediators. This novel cross-talk may play a critical role in mediating the amplification of pathological processes in the kidney presenting a therapeutic basis to manipulate the cascade of inflammatory responses.

# References

ANDREW, P.J., HARANT, H. & LINDLEY, I.J. (1995). Nitric oxide regulates IL-8 expression in melanoma cells at the transcriptional level. *Biochem. Biophys. Res. Commun.*, **214**, 949 – 956.

BAUD, L., FOUQUERAY, B., PHILIPPE, C. & ARDAILLOU, R. (1992). Reactive oxygen species as glomerular autacoids. *J. Am. Soc. Nephr.*, **2** (10 Suppl), S132 – S138.

BREDT, D.S. & SNYDER, S.H. (1994). Nitric oxide: a physiologic messenger molecule. *Annu. Rev. Biochem.*, **63**, 175 – 195.

CATTELL, V. & COOK, T. (1993). Nitric oxide: role in the physiology and pathology of the glomerulus. *Exp. Nephrol.*, **1**, 265 – 280.

CATTELL, V. & COOK, T. (1995). The nitric oxide pathway in glomerulonephritis. *Curr. Opin. Nephrol. Hypertens.*, **4**, 359 – 364.

FEELISCH, M. (1998). The use of nitric oxide donors in pharmacological studies. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **358**, 113 – 122.

GREEN, L.C., WAGNER, D.A., GLOGOWSKI, J., SKIPPER, P.L., WISHNOK, J.S. & TANNENBAUM, S.R. (1982). Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. *Anal. Biochem.*, **126**, 131 – 138.

HACK, C.E., WOLBINK, G.-J., SCHALKWIJK, C., SPEIJER, H., HERMENS, W.T. & VAN DEN BOSCH, H. (1997). A role for secretory phospholipase A$_2$ and C-reactive protein in the removal of injured cells. *Immunology Today*, **18**, 111 – 115.

HARA, S., KUDO, I., KOMATANI, T., TAKAHASHI, K., NAKATANI, Y., NATORI, Y., OHSHIMA, M. & INOUE, K. (1995). Detection and purification of two 14 kDa phospholipase A$_2$ isoforms in rat kidney: their role in eicosanoid synthesis. *Biochim. Biophys. Acta*, **1257**, 11 – 17.

HUWILER, A., STAUDT, G., KRAMER, R.M. & PFEILSCHIFTER, J. (1997). Cross-talk between secretory phospholipase A$_2$ and cytosolic phospholipase A$_2$ in rat renal mesangial cells. *Biochim. Biophys. Acta*, **1348**, 257 – 272.

KANEMATSU, M., IKEDA, K. & YAMADA, Y. (1997). Interaction between nitric oxide synthase and cyclooxygenase pathways in osteoblastic MC3T3-E1 cells. *J. Bone Miner. Res.*, **12**, 1789 – 1796.

KRAMER, R.M. & SHARP, J.D. (1997). Structure, function and regulation of Ca2 + -sensitive cytosolic phospholipase A2 (cPLA2). *FEBS Lett.*, **410**, 49 – 53.

MARAGOS, C.M., MORLEY, D., WINK, D.A., DUNAMS, T.M., SAAVEDRA, J.E., HOFFMAN, A., BOVE, A.A., ISASAC, L., HRABIE, J.A. & KEEFER, L.K. (1991). Complexes of NO with nucleophiles as agents for the controlled biological release of nitric oxide. Vasorelaxant effects. *J. Med. Chem.*, **34**, 3242 – 3247.

MÄRKI, F. & FRANSON, R. (1986). Endogenous suppression of neutral-active and calcium-dependent phospholipase A$_2$ in human polymorphonuclear leukocytes. *Biochim. Biophys. Acta*, **879**, 149 – 156.

MOLLACE, V., COLASANTI, M., MUSCOLI, C., LAURO, G.M., IANNONE, M., ROTIROTI, D. & NISTICO, G. (1998). The effect of nitric oxide on cytokine-induced release of PGE$_2$ by human cultured astroglial cells. *Br. J. Pharmacol.*, **124**, 742 – 746.

MONCADA, S. & HIGGS, A. (1993). The l-arginine-nitric-oxide pathway. *N. Eng. J. Med.*, **329**, 2002 – 2011.

MÜHL, H. & PFEILSCHIFTER, J. (1995). Amplification of nitric oxide synthase expression by nitric oxide in interleukin 1 beta-stimulated rat mesangial cells. *J. Clin. Invest.*, **85**, 1941 – 1946.

MÜHL, H., SANDAU, K., BRÜNE, B., BRINER, V. & PFEILSCHIFTER, J. (1996). Nitric oxide donors induce apoptosis in glomerular mesangial cells, epithelial cells and endothelial cells. *Eur. J. Pharmacol.*, **317**, 137 – 149.

MURAKAMI, M., SHIMBARA, S., KAMBE, T., KUWATA, H., WINSTEAD, M.V., TISCHFIELD, J.A. & KUDO, I. (1998). The functions of five distinct mammalian phospholipase A$_2$s in regulating arachidonic acid release. Type IIa and type V secretory phospholipase A$_2$s are functionally redundant and act in concert with cytosolic phospholipase A$_2$. *J. Biol. Chem.*, **273**, 14411 – 14423.

PFEILSCHIFTER, J. (1994). Mesangial cells orchestrate inflammation in the renal glomerulus. *News Physiol. Sci.*, **9**, 271 – 276.

PFEILSCHIFTER, J. (1995). In: *Molecular approaches to pathophysiology*, Glaser, K.B., Vada P, eds. pp. 25 – 51. New York: CRC Press.

PFEILSCHIFTER, J., KURTZ, A. & BAUER, C. (1984). Activation of phospholipase C and prostaglandin synthesis by [arginine]vasopressin in cultures. *Biochem. J.,* **223,** 855–859.

PFEILSCHIFTER, J., ROB, P., MÜLSCH, A., FANDREY, J., VOSBECK, K. & BUSSE, R. (1992). Interleukin 1$\beta$ and tumor necrosis factor $\alpha$ induce a macrophage-type of nitric oxide synthase in rat renal mesangial cells. *Eur. J. Biochem.,* **203,** 251–255.

PFEILSCHIFTER, J., SCHALKWIJK, C., BRINER, V.A. & VAN DEN BOSCH, H. (1993). Cytokine-stimulated secretion of group II phospholipase A$_2$ by rat mesangial cells. *J. Clin. Invest.,* **92,** 2516–2523.

PFEILSCHIFTER, J. & SCHWARZENBACH, H. (1990). Interleukin 1 and tumour necrosis factor stimulate cGMP in rat renal mesangial cells. *FEBS Lett.,* **273,** 185–187.

PRUZANSKI, W., STEFANSKI, E., DE BEER, F.C., DE BEER, M.C., VADAS, P., RAVANDI, A. & KUKSIS, A. (1998). Lipoproteins are substrates for human secretory group IIA phospholipase A$_2$. Preferential hydrolysis of acute phase HDL. *J. Lipid Res.,* **39,** 2150–2160.

REMICK, D.G. & VILLARETE, L. (1996). Regulation of cytokine gene expression by reactive oxygen and reactive nitrogen intermediates. *J. Leukoc. Biol.,* **59,** 471–475.

SALVEMINI, D. (1997). Regulation of cyclooxygenase enzymes by nitric oxide. *Cell. Mol. Life Sci.,* **53,** 576–582.

SALVEMINI, D., MISKO, T.P., MASFERRER, J.L., SEIBERT, K., CURRIE, M.G. & NEEDLEMAN, P. (1993). Nitric oxide activates cyclooxygenase enzymes. *Proc. Natl. Acad. Sci. USA,* **90,** 7240–7244.

SALVEMINI, D., SEIBERT, K., MASFERRER, J.L., MISKO, T.P., CURRIE, M.G. & NEEDLEMAN, P. (1994). Endogenous nitric oxide enhances prostaglandin production in a model of renal inflammation. *J. Clin. Invest.,* **93,** 1940–1947.

SAMBROOK, J., FROTSCJ, J. & MANIATIS, T. (1989). Molecular cloning: a Laboratory Manual, Cold Spring Harbor, Cold Spring Harbor Laboratory Press, New York.

SANDAU, K., PFEILSCHIFTER, J. & BRÜNE, B. (1997). The balance between nitric oxide and superoxide determines apoptotic and necrotic death of rat mesangial cells. *J. Immunol.,* **158,** 4938–4946.

SAUTEBIN, L., IALENTI, A., IANARO, A. & DI ROSA, M. (1995). Modulation by nitric oxide of prostaglandin biosynthesis in the rat. *Br. J. Pharmacol.,* **114,** 323–328.

SWIERKOSZ, T.A., MITCHELL, J.A., WARNER, T.D., BOTTING, R.M. & VANE, J.R. (1995). Co-induction of nitric oxide synthase and cyclo-oxygenase: interactions between nitric oxide and prostanoids. *Br. J. Pharmacol.,* **114,** 1335–1342.

TETSUKA, T., DAPHNA-IKEN, D., MILLER, B.W., GUAN, Z., BAIER, L.D. & MORRISON, A.R. (1996). Nitric oxide amplifies interleukin 1-induced cyclooxygenase-2 expression in rat mesangial cells. *J. Clin. Invest.,* **97,** 2051–2056.

VADAS, P. & PRUZANSKI, W. (1986). Role of secretory phospholipases A$_2$ in the pathobiology of disease. *Lab. Invest.,* **55,** 391–404.

VADAS, P. & PRUZANSKI, W. (1993). Induction of group II phospholipase A$_2$ expression and pathogenesis of the sepsis syndrome. *Circ. Shock,* **39,** 160–167.

VAN SCHAIK, R.H.N., VERHOEVEN, N.M., NEIJS, F.W., AARSMAN, A.J. & VAN DEN BOSCH, H. (1993). Cloning of the cDNA coding for 14 kDa group II phospholipase A$_2$ from rat liver. *Biochim. Biophys. Acta,* **1169,** 1–11.

VILLARETE, L.H. & REMICK, D.G. (1997). Nitric oxide regulation of interleukin-8 gene expression. *Shock,* **7,** 29–35.

WATKINS, D.N., GARLEPP, M.J. & THOMPSON, P.J. (1997). Regulation of the inducible cyclo-oxygenase pathway in human cultured airway epithelial (A549) cells by nitric oxide. *Br. J. Pharmacol.,* **121,** 1482–1488.