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Modulation by nitric oxide of prostaglandin biosynthesis in the rat

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1 Modulation of prostaglandin biosynthesis *in vivo* by either exogenous or endogenous nitric oxide (NO) has been studied in the rat using arachidonic acid (AA)-induced paw oedema and measuring both the foot volume and the amount of 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), the stable metabolite of prostacyclin (PGI_2), in the oedematous fluid recovered from inflamed paws.

2 Paw injections of 150 or 300 nmol of AA were virtually inactive whereas 600 nmol produced a moderate oedema which was greatly reduced by the NO synthase inhibitor L-N^G-nitro arginine methyl ester (L-NAME, 100 nmol/paw) and the NO scavenger haemoglobin (Hb, 30 μmol/paw), but unaffected by the inhibitor of the soluble guanylate cyclase, methylene blue (Mb, 3 μmol/paw) and L-arginine (15 μmol/paw).

3 The NO-donors (10 μmol/paw) 3-morpholino-sydnonimine-hydrochloride (SIN-1), S-nitroso-N-acetyl-D, L-penicillamine (SNAP) and sodium nitroprusside (SNP) significantly potentiated the paw oedema induced by AA (300 nmol/paw).

4 SIN-1 (2.5, 5 and 10 μmol/paw) produced a significant dose-dependent increase of the oedema induced by AA which was correlated with increased amounts of 6-keto-$PGF_{1\alpha}$ in the fluid recovered from inflamed paws.

5 Both oedema and prostaglandin biosynthesis induced by the combination AA + SIN-1 were greatly suppressed by either Hb (30 μmol/paw) or indomethacin (3 μmol/paw or 5 mg kg^{-1} s.c.) but unaffected by Mb (3 μmol/paw).

6 In LPS-treated rats (6 mg kg^{-1}, i.p.) doses of AA inactive in normal animals produced a remarkable oedema which was reduced by L-NAME or Hb, unaffected by Mb and increased by L-arginine.

7 These results demonstrate that NO increases prostaglandin biosynthesis *in vivo* through a guanosine 3':5'-cyclic monophosphate (cyclic GMP)-independent mechanism and suggest that the interaction between NO synthase and cyclo-oxygenase (COX) pathways may represent an important mechanism for the modulation of the inflammatory response.

Keywords: Arachidonic acid; endotoxin; nitric oxide; NO donors; oedema; prostaglandin biosynthesis

Introduction

Nitric oxide (NO) is generated from the terminal guanidino nitrogen atom(s) of the amino acid L-arginine by the enzyme NO synthase (NOS; for review see Moncada *et al.*, 1991). At least two types of NOS have been so far identified. One is constitutive (cNOS), Ca^{2+}/calmodulin-dependent and releases NO for short periods in response to receptor stimulation. The other enzyme is inducible (iNOS), Ca^{2+}-independent and, once expressed, generates NO for long periods. Both enzymes are inhibited by certain L-arginine analogues (Rees *et al.*, 1990), whereas glucocorticoids inhibit the induction of iNOS (Di Rosa *et al.*, 1990). The cNOS has been identified in vascular endothelium and brain, while iNOS is expressed after stimulation with bacterial lipopolysaccharide (LPS) or some cytokines in endothelial cells, smooth muscle cells, polymorphonuclear leucocytes and macrophages.

NO generated by cNOS is responsible for a variety of biological actions which include endothelium-dependent relaxation, inhibition of platelet aggregation and cell-to-cell communication in the central nervous system. These actions are a result of NO acting as the endogenous stimulator of soluble guanylate cyclase. NO produced in large quantities by iNOS plays an important role in host-defence response, in the pathogenesis of endotoxin shock and in autoimmune tissue destruction (Hibbs *et al.*, 1988; Kilbourn *et al.*, 1990; Kolb *et al.*, 1991).

We have shown that the L-arginine:NO pathway is involved in both acute and chronic inflammation since endogenous NO increases oedema formation and exacerbates adjuvant arthritis in the rat (Ialenti *et al.*, 1992; 1993). We have also shown that depending on the time course or on the type of inflammation, NO may be predominantly generated either by cNOS or by iNOS.

Prostaglandins play a major role as mediators of the inflammatory response. Prostaglandins are generated from arachidonic acid (AA) by the enzyme cyclo-oxygenase (COX). Like NOS, COX has been found in two forms, namely the constitutive, widely occurring enzyme (COX-1) and the inducible isoform (COX-2), which is expressed in endothelial cells and macrophages after stimulation with LPS or some cytokines (Maier *et al.*, 1990; Fu *et al.*, 1990; Masferrer *et al.*, 1990; 1992). Recently it has been reported that, in the mouse macrophage cell line RAW264.7, NO activates both COX-1 and COX-2 by a guanosine 3':5'cyclic monophosphate (cyclic GMP)-independent mechanism (Salvemini *et al.*, 1993). Furthermore we have shown that prostacyclin biosynthesis in the lung of LPS-treated rats is increased by L-arginine and decreased by NOS inhibitors suggesting that endogenous NO may modulate prostanoid production (Sautebin & Di Rosa, 1994).

The role of NO in inflammation may, therefore, depend not only on its own ability to increase vascular permeability and oedema formation, possibly by increasing local blood flow, but also on the concomitant enhancement of proinflammatory prostaglandin biosynthesis due to the activation of COX. In this study we have investigated *in vivo* the modulation of prostaglandin biosynthesis by either exogenous or

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endogenous NO using the arachidonic acid (AA)-induced rat paw oedema and measuring both the foot volume and the amounts of 6-keto-$PGF_{1\alpha}$, the stable metabolite of prostacyclin (PGI_2), in the oedematous fluid recovered from inflamed paws.

Methods

Paw oedema was induced in male Wistar rats (140–160 g) by subplantar injection of 0.1 ml saline containing AA or other agents which were given alone or in combination. AA was dissolved in absolute ethanol before dilution in saline. Other agents injected into the rat paw were: the NO donors 3-morpholino-sydnonimine-hydrochloride (SIN-1), S-nitroso-N-acetyl-D, L-penicillamine (SNAP) and sodium nitroprusside (SNP), the NOS inhibitor L-N^G-nitro arginine methyl ester (L-NAME), the NO scavenger haemoglobin (Hb), the inhibitor of soluble guanylate cyclase methylene blue (Mb), L-arginine, D-arginine, linoleic acid and indomethacin. In some experiments indomethacin was also given s.c. 1 h before paw injections.

The volume of the paw was measured by a plethysmometer (Basile, Italy) immediately after the injection as previously described (Di Rosa and Willoughby, 1971). Subsequent readings of the volume of the same paw were carried out at 30 min intervals up to 120 min and compared to the initial readings. The increase in paw volume was taken as oedema volume. In some experiments rats were killed in an atmosphere of CO_2 immediately after the readings at 90 min. Each inflamed paw was cut at the level of the calcaneus using a guillotine and gently spun (250 *g* for 15 min) in order to recover a sample of the oedamatous fluid. The blood was removed by filtering the samples through a 10,000 Mr cut off filter (Centricon 10, Amicon). The amount of 6-keto-$PFG_{1\alpha}$ in the sample was measured by radioimmunoassay (McLouf, 1982).

The total amount (TA) of 6-keto-$PGF_{1\alpha}$ present in the entire oedematous fluid of each paw was calculated as follows:

$$TA = \frac{\text{6-keto-PGF}_{1\alpha} \text{ in the sample (pmol)} \times \text{paw oedema volume (ml)}}{\text{sample volume (ml)}}$$

and expressed as pmol of 6-keto-$PGF_{1\alpha}$ per paw.

In some experiments the oedema was induced in rats treated with bacterial Salmonella typhosa lipopolysaccharide (LPS, 6 mg kg^{-1}) given i.p. 6 h prior to the paw injections.

Some control or LPS-treated rats did not receive the paw injection as they were used for the assay of the iNOS in the lung. These animals were anaesthetized with urethane (1.3 g kg^{-1}, i.p.) 6 h after LPS injection and lungs removed and washed. The activity of iNOS in the soluble lung extracts was evaluated by a radioisotopic technique based on the conversion of L-[U-^{14}C]-arginine to [U-^{14}C]-citrulline (Salter *et al.*, 1991). Proteins were assayed according to the method of Bradford (1976).

SIN-1 was a gift from A.G. Casella (Frankfurt), SNAP was provided by Dr H. Hodson, Dept. of Medicinal Chemistry, Wellcome Research Labs. (Beckenham, U.K.). LPS was obtained from Difco, the anti-6 keto $PGF_{1\alpha}$ serum was a gift from Dr G. Folco (Milan), L-[U-^{14}C] arginine and 6-keto [5,8,9,11,12,14,15 (n) -^{3}H] $PGF_{1\alpha}$ were from Amersham. All other compounds were from Sigma.

Data are expressed as means ± s.e.mean. Comparisons were made by an unpaired Student's two-tailed *t* test. The level of statistically significant difference was defined as $P<0.05$.

Results

Exogenous NO increases AA-induced oedema

In preliminary experiments we established that injection into the rat paw of L-NAME (up to 100 nmol), Hb (up to 30 μmol), Mb (up to 3 μmol), L- or D-arginine (up to 15 μmol) did not produce any detectable oedema. Injection of SIN-1 produced a moderate dose-related oedema with a peak response occurring at 60 min. Thus at this time the oedema induced by 2.5, 5 and 10 μmol/paw was 0.15 ± 0.02 ml (n = 6), 0.24 ± 0.02 ml (n = 6) and 0.33 ± 0.01 ml (n = 6), respectively. When SIN-1 (10 μmol) was coinjected with 10 μmol Hb the oedema was greatly reduced (data not shown) while it was completely suppressed by 30 μmol Hb (Figure 1). Coinjection of Mb (3 μmol) greatly reduced the SIN-1 oedema at 30 and 60 min and virtually abolished it at 90 and 120 min whereas the oedema resulted unaffected by coinjection of L-NAME (100 nmol) or indomethacin (3 μmol). Injection of SNAP (10 μmol) or SNP (10 μmol) produced a slight oedema with a peak response also occurring at 60 min (0.18 ± 0.03 ml, n = 6 and 0.12 ± 0.02 ml, n = 5, respectively). At 90 min the oedemas induced by SNAP and SNP were 0.12 ± 0.01 ml (n = 6) and 0.07 ± 0.01 ml (n = 5), respectively. Both SNAP- and SNP-induced oedemas were suppressed by Hb and Mb and unaffected by L-NAME and indomethacin (not shown). These results suggest that the oedema induced by the NO donors SIN-1, SNAP and SNP was actually due to exogenous NO acting through a cyclic GMP-dependent mechanism.

Injection into the rat paw of 300 nmol AA produced a very slight oedema. However, when the same amount of AA was coinjected with 10 μmol SIN-1, the resulting oedema was enormously increased, with a peak response occurring at 90 min (Figure 2). The oedema induced by AA (300 nmol) at 90 min was also significantly potentiated when it was coinjected with 10 μmol SNAP (0.20 ± 0.02 ml, n = 6, $P<0.01$) or SNP (0.12 ± 0.01 ml, n = 5, $P<0.05$). Interestingly no interaction occurred between linoleic acid (300 nmol) and SIN-1 (10 μmol) since the resulting oedema was superimposable on the one produced by SIN-1 given alone (Figure 2).

The oedema induced by AA + SIN-1 was virtually abolished when these two agents were given together with 30 μmol Hb whereas it was unaffected when they were given with 3 μmol Mb or 100 nmol L-NAME (Figure 3). Since the SIN-1 mediated increase of AA-induced oedema was abolished by the NO scavenger, Hb and unaffected by the guanylate cyclase inhibitor, Mb it appears that NO was acting through a cyclic GMP-independent mechanism. Indomethacin (3 μmol) produced a marked inhibition of AA + SIN-1 oedema (Figure 3) and it was equally effective when given s.c. at 5 mg kg^{-1} 1 h prior the paw injection of AA + SIN-1, for it reduced the oedema by about 50% throughout the time course of the reaction, suggesting that PGs were actually generated into the inflamed paw.

When various dose-combinations of AA + SIN-1 were used, the resulting oedema greatly increased in a dose-related fashion (Figure 4).

We determined the amount of 6-keto-$PGF_{1\alpha}$, the stable metabolite of PGI_2, in the oedematous fluid recovered from the inflamed paw of rats (Table 1). The results of these experiments show that SIN-1 dose-dependently increased the amount of PGI_2 produced by the tissues of the inflamed paw. The SIN-1-mediated increase of PGI_2 was significantly reduced by Hb and indomethacin but was unaffected by Mb.

Endogenous NO increases AA-induced oedema

The oedema produced by paw injection of 150 or 300 nmol AA was virtually undetectable, while 600 nmol induced a measurable oedema with a peak response occurring at 90 min (Figure 5). The AA-induced oedema was greatly inhibited by

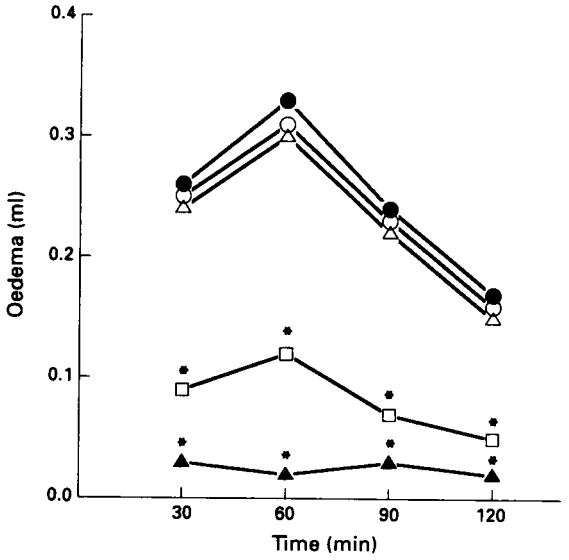

Figure 1 Effect of N^G-nitro-L-arginine methyl ester (L-NAME) 100 nmol (△), indomethacin 3 μmol (○), methylene blue 3 μmol (□) and haemoglobin 30 μmol (▲) on rat paw oedema induced by 3-morpholino-sydnonimine (SIN-1) 10 μmol. The oedema induced by 10 μmol SIN-1 given alone (control) is shown by (●). Data are expressed as means from 5–6 animals. Standard errors (not shown) were always less than 15% of the respective means. $^*P<0.01$ *vs* control group.

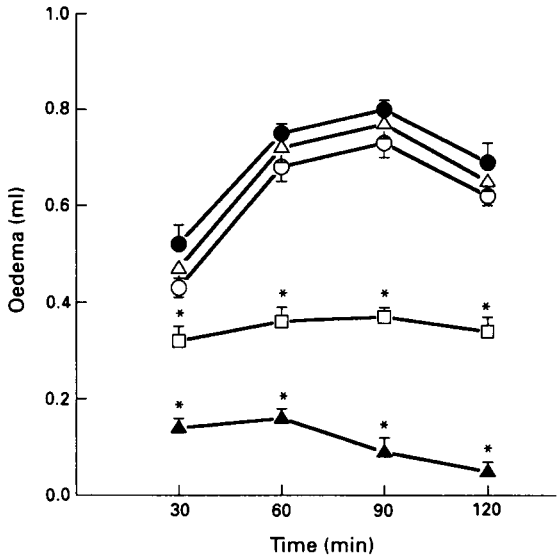

Figure 3 Effect of methylene blue (Mb) 3 μmol (△), N^G-nitro-L-arginine methyl ester (L-NAME) 100 nmol (○), indomethacin 3 μmol (□) and haemoglobin (Hb) 30 μmol (▲) on the oedema induced by the combination of arachidonic acid (AA) 300 nmol + 3-morpholino-syndnonimine (SIN-1) 10 μmol. The oedema induced by the combination AA + SIN-1 (control) is shown by (●). Data are expressed as means ± s.e.mean from 5–6 animals. $^*P<0.01$ *vs* control group.

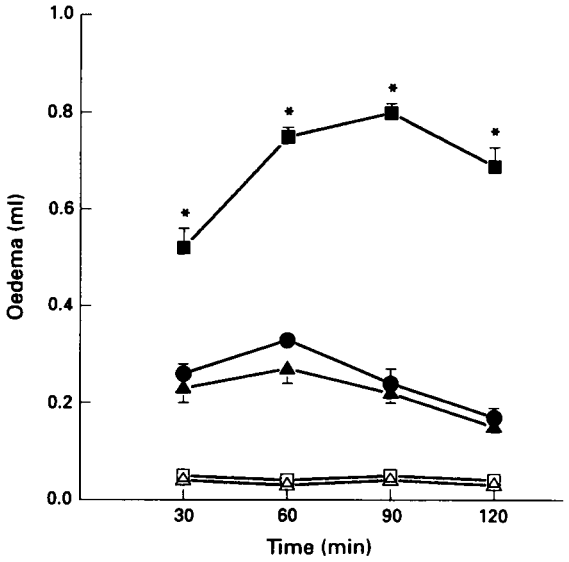

Figure 2 Rat paw oedema induced by 300 nmol arachidonic acid (AA) given alone (□) or in combination with 10 μmol 3-morpholino-sydnonimine (SIN-1) (■) and by 300 nmol linoleic acid given alone (△) or in combination with 10 μmol SIN-1 (▲). The oedema induced by 10 μmol SIN-1 alone (control) is shown by (●). Data are expressed as means ± s.e.mean from 5–6 animals. Standard errors less than 5% of the respective means are not shown because they are covered by the symbols. $^*P<0.01$ *vs* control group.

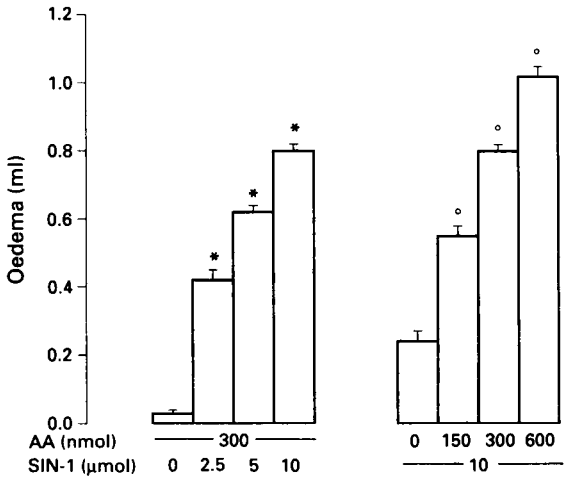

Figure 4 Rat paw oedema induced by various combinations of arachidonic acid (AA) and 3-morpholino-sydnonimine (SIN-1). Each column represents the mean value ± s.e.mean (bar) of the oedema occurring at 90 min. ($n=5–6$). $^*P<0.01$ *vs* 300 nmol AA given alone; $^\circ P<0.01$ *vs* 10 μmol SIN-1 given alone.

coinjection of indomethacin (3 μmol), showing that the response was due in part to COX activity. When AA (600 nmol) was coinjected with L-NAME (100 nmol) or Hb (30 μmol) the oedema was greatly reduced whereas it was not modified by Mb (3 μmol) showing that endogenous NO modulates AA-induced oedema by a cyclic GMP-independent mechanism. However when L-arginine (15 μmol) was coinjected with AA (600 nmol) no interaction occurred between these two agents for the resulting oedema was superimposable on the response of AA given alone (Figure 5). Since it is known that an excess of L-arginine does not increase the activity of the cNOS this result suggests that, in our conditions, it is the constitutive enzyme that generates the NO responsible of the enhanced formation of PGs from AA.

Therefore we decided to investigate the effect of L-arginine on AA-induced oedema in LPS-treated rats in which the iNOS is also expressed. The iNOS activity was measured in the lung since it has been shown that this tissue expresses

Table 1 Alterations in amounts of 6-keto-$PGF_{1\alpha}$ in rat paw oedema induced by arachidonic acid (AA, 300 nmol) given alone or coinjected with various agents

Group	*Agents* (μmol)	*6-keto-$PGF_{1\alpha}$* (pmol per paw)	n
1	AA	89 ± 12	5
2	SIN-1 10	104 ± 14	4
3	AA + SIN-1 2.5	189 ± 29†	4
4	AA + SIN-1 5	226 ± 15†	4
5	AA + SIN-1 10	288 ± 8†	7
6	AA + SIN-1 10 + Hb 30	92 ± 13*	4
7	AA + SIN-1 10 + Mb 3	254 ± 51	3
8	AA + SIN-1 10 + indomethacin 3	163 ± 25*	4
9	AA + SIN-1 10 + indomethacin 5 mg kg^{-1}	145 ± 12*	4

The amounts of 6-keto-$PGF_{1\alpha}$ were measured by radioimmunoassay in samples of the oedematous fluid recovered from the inflamed paws 90 min after the injections and expressed as total amount present in each paw. In group 9 indomethacin was given s.c. 1 h before paw injection. †$P<0.01$ *vs* group 1; *$P<0.01$ *vs* group 5. For abbreviations, see text.

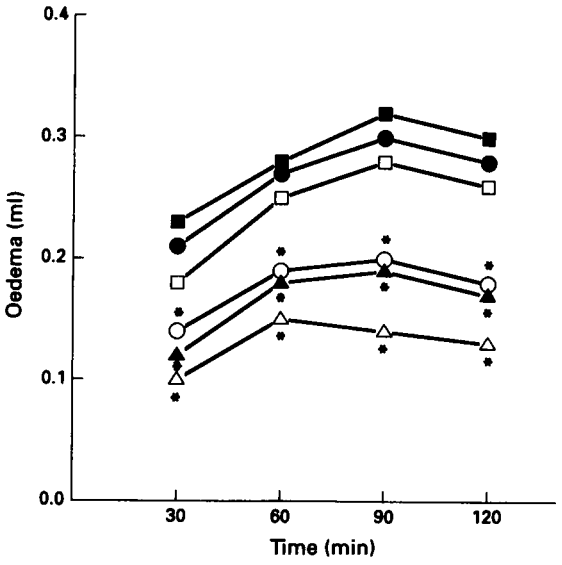

Figure 5 Effect of L-arginine 15 μmol (■), methylene blue 3 μmol (□), indomethacin 3 μmol (○), haemoglobin (Hb) 30 μmol (▲) and N^G-nitro-L-arginine methyl ester (L-NAME) 100 nmol (△) on rat paw oedema induced by 600 nmol arachidonic acid (AA). The oedema induced by 600 nmol AA given alone (control) is shown by (●). Data are expressed as means from 5–6 animals. Standard errors (not shown) were always less than 15% of the respective means. *$P<0.01$ *vs* control group.

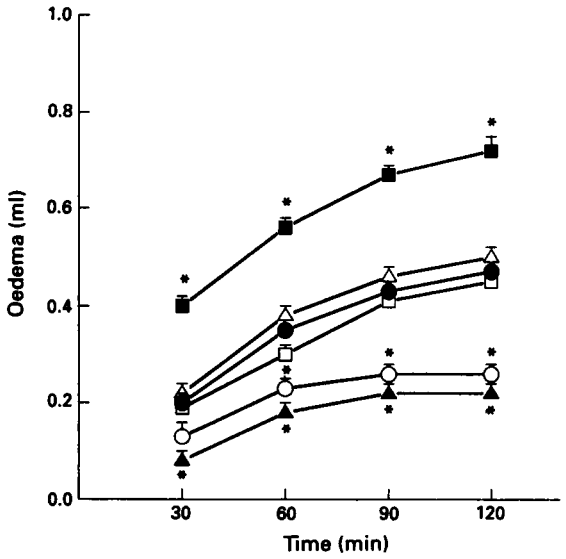

Figure 6 Effect of L-arginine 15 μmol (■), D-arginine 15 μmol (△), methylene blue 3 μmol (□), haemoglobin 30 μmol (○) and L-NAME 100 nmol (▲) on paw oedema induced by 300 nmol arachidonic acid (AA) in LPS-treated rats. The oedema induced by 300 nmol AA given alone (control) is shown by (●). Animals were treated with LPS (6 mg kg^{-1}, i.p.) 6 h before paw injection. Data are expressed as means ± s.e.mean from 5–6 animals. Standard errors less than 5% of the respective means are not shown because they are covered by the symbols. *$P<0.01$ *vs* control group.

Table 2 Alterations in amounts of 6-keto-$PGF_{1\alpha}$ in paw oedema induced in LPS-treated rats by arachidonic acid (AA, 300 nmol) given alone or coinjected with various agents

Group	*Agents* (μmol)	*6-keto-$PGF_{1\alpha}$* (pmol per paw)	n
1	AA (alone)	289 ± 21	7
2	AA + L-arginine 15	384 ± 54*	3
3	AA + D-arginine 15	286 ± 12	3
4	AA + L-NAME 0.1	102 ± 4**	4
5	AA + Hb 30	148 ± 8**	4
6	AA + Mb 3	278 ± 18	3

The amounts of 6-keto-$PGF_{1\alpha}$ were measured by radioimmunoassay in samples of the oedematous fluid recovered from the inflamed paws 90 min after the injections and expressed as the total amount present in each paw. Lipopolysaccharide (LPS, 6 mg kg^{-1}) was given i.p. 6 h before paw injection. *$P<0.05$, **$P<0.01$ *vs* group 1. For abbreviations, see text.

iNOS in LPS-challenged rats (Knowles *et al.*, 1990). The iNOS activity in the lung of control rats was undetectable whereas it was substantially induced in lungs removed 6 h after rats were treated with LPS (6 mg kg^{-1}, i.p.). Thus in our experimental conditions the conversion rate of L-arginine to L-citrulline was 14.2 ± 1.1 ($n = 5$) pmol min^{-1} per mg of lung proteins.

In LPS-treated rats, paw injection of 300 nmol AA induced a long lasting oedema (Figure 6) which was significantly greater when compared to the almost undetectable paw swelling produced by the same amount of AA given to normal rats (Figure 2). AA-induced oedema in LPS-treated rats was greatly reduced by coinjection of either L-NAME (100 nmol) or Hb (30 μmol) whereas it was unaffected by Mb (3 μmol). Interestingly, in LPS-treated rats, unlike normal rats (Figure 4), coinjection of L- but not of D-arginine (15 μmol) significantly enhanced AA-induced oedema. This enhancement probably depends on the increased amounts of NO generated by the iNOS, the enzyme isoform whose activity, unlike that of cNOS, is stimulated by an excess of substrate.

The amounts of 6-keto-$PGF_{1\alpha}$ found in the inflamed paws of LPS-treated rats correlated well with the severity of the oedema induced by AA alone or in combination with the various agents (Table 2).

These results suggest that the enhancement of AA-induced oedema in LPS-treated rats, although it may depend in part on the coexpression of COX-2 which increases the conversion of AA to prostaglandins is due to endogenous NO which regulates prostaglandin biosynthesis.

Discussion

In this study we have shown that both exogenous and endogenous NO greatly increase AA-stimulated biosynthesis of prostaglandins. The AA-induced rat paw oedema was significantly increased by the NO-donors SNAP, SNP and SIN-1, the active metabolite of molsidomine (Noack & Feelisch, 1989). SIN-1 produced a moderate oedema due to the release of exogenous NO acting through a cyclic GMP-dependent mechanism since it was suppressed by the NO scavenger, Hb and by the soluble guanylate cyclase inhibitor, Mb. This result is in agreement with our previous results showing that endogenous NO has a role in the regulation of increased vascular permeability and oedema formation, possibly by increasing local blood flow (Ialenti *et al.*, 1992).

Inactive doses of AA, but not of linoleic acid, when injected concomitantly with SIN-1, produced a marked paw oedema which was correlated with increased amounts of 6-keto-$PGF_{1\alpha}$, the stable metabolite of PGI_2, in the fluid recovered from inflamed paws.

Both oedema and PGI_2 biosynthesis induced by the combination AA + SIN-1 were greatly suppressed by either the NO scavenger Hb or the COX inhibitor indomethacin but remained unaffected by Mb, an inhibitor of the soluble guanylate cyclase. These results demonstrate that exogenous NO released by SIN-1 increases AA-stimulated prostaglandin biosynthesis by activating COX through a cyclic GMP-independent mechanism which, as has been suggested (Salvemini *et al.*, 1993), possibly depends on a direct interaction of NO with the iron-haeme centre of COX.

Our results suggest that endogenous NO, acting via the same cyclic GMP-independent mechanism, increases AA-stimulated prostaglandin biosynthesis in both normal rats, in which only cNOS and COX-1 are present, and in LPS-treated rats, in which the iNOS and COX-2 are also expressed.

In normal rats, the oedema induced by a high dose of AA was reduced by L-NAME, Hb or indomethacin, unaffected by Mb and, as expected, it was not modified by L-arginine since it is known that an excess of substrate does not increase the activity of cNOS. In LPS-treated rats, a low dose of AA, which was inactive in normal animals, induced a very marked oedema. This oedema correlated with increased prostaglandin biosynthesis which depended not only on the activity of COX-2 but also on the generation of large amounts of NO by the iNOS.

In LPS-treated animals, the AA-induced oedema was reduced by L-NAME, Hb and indomethacin, unaffected by Mb and, unlike in normal rats, was increased by L- but not by D-arginine. The LPS-induced iNOS can be stimulated by an excess of substrate, so that larger amounts of NO are generated which presumably in turn activated COX. These findings are also supported by our previous report showing that in the lung of LPS-treated rats PG generation was increased by L-arginine and decreased by NOS inhibitors (Sautebin & Di Rosa, 1994).

Our results demonstrate that NO increases AA-stimulated biosynthesis of prostaglandin *in vivo* by a cyclic GMP-independent mechanism and are in agreement with recent data showing that NO activates COX enzymes *in vitro* (Salvemini *et al.*, 1993).

The findings of this study may have physiological, pathological and therapeutic relevance. In normal rats, either inhibition of cNOS by L-NAME or inactivation of NO by Hb result in a reduced biosynthesis of prostaglandins suggesting that even in physiological conditions COX activity may be permanently stimulated by endogenous NO. Thus the synergistic inhibitory effects of NO and PGI_2 on vascular smooth muscle tone and platelets aggregation may depend not only on the concomitant elevation in target cells of the respective second messengers, cyclic GMP and cyclic AMP, but also on the increased biosynthesis of PGI_2 induced by NO interacting directly with COX through a cyclic GMP-independent mechanism.

In addition it is possible that the vasoconstriction and elevation of systemic blood pressure induced by NOS inhibitors (Gardiner *et al.*, 1990) could be due not only to the removal of endogenous NO but also to a consequent reduced production of vasodilator prostaglandins. This last mechanism might be of interest for a clearer understanding of the beneficial effects of NOS inhibitors observed in animals and man in the treatment of vascular dysfunctions occurring in cytokine-induced hypotension or LPS-induced shock (Kilbourn *et al.*, 1990; Thiemermann *et al.*, 1990; Petros & Vallance, 1993).

The interaction between the NO and COX pathways should also be relevant in acute and chronic inflammation which appear to be modulated by NO since in both types of process the degree of lesion was reduced by NOS inhibitors and exacerbated by L-arginine (Ialenti *et al.*, 1992; 1993). Thus in a pathological condition like inflammation in which the iNOS and COX-2 enzymes are coexpressed, there is an NO-mediated increase of proinflammatory prostaglandins that may amplify the inflammatory response.

We have shown that PGE_2 and iloprost (a stable analogue of PGI_2) both at nanomolar concentrations inhibited the LPS-stimulated induction of iNOS in the murine macrophage cell line J774 (Marotta *et al.*, 1992). Furthermore, PGE_2 and PGI_2 have been found to inhibit the release of TNF-α from activated mouse peritoneal macrophages (Marcinkiewicz, 1991), rat alveolar macrophages (Mohr *et al.*, 1992) and human peripheral blood mononuclear cells (Haynes *et al.*, 1992). Since it is known that this cytokine induces iNOS, these findings support the hypothesis that the NO-mediated overproduction of prostaglandins may also act as a negative feedback preventing excessive or prolonged induction of iNOS (Figure 7).

In conclusion we have demonstrated that NO modulates PG biosynthesis *in vivo*, in both physiological and pathological conditions, and suggest that not only does NO activate COX enzymes, as has been proposed by Salvemini *et al.* (1993), but also that in some pathological conditions, such as inflammation or sepsis, in which both iNOS and COX-2 are present, some products of COX may downregulate the expression of iNOS.

Finally it would be interesting to ascertain whether the therapeutic efficacy of nitrovasodilators, which is due to their release of exogenous NO, depends entirely on the activation of soluble guanylate cyclase or if it may be due, at least in part, to the stimulation of COX activity, via a cyclic GMP-independent mechanism, which in turn leads to increased production of vasodilator prostaglandins.

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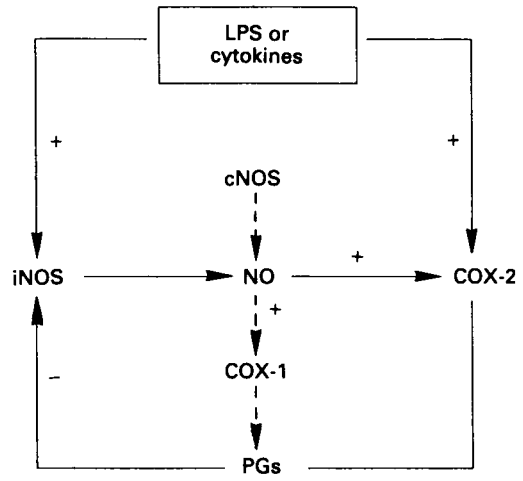

Figure 7 Model for the mutual interactions between NO synthase (NOS) and cyclo-oxygenase (COX) systems in physiological (dotted arrows) or pathological conditions (solid arrows). For details see Discussion.

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