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Regulation of Prostaglandin E_2 Production by the Superoxide Radical and Nitric Oxide in Mouse Peritoneal Macrophages

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The purpose of this study was to elucidate the role of NO and O_2^- on enzymatic components of cyclooxygenase (COX) pathway in peritoneal macrophages. Activation of murine peritoneal macrophages by lipopolysaccharides (LPS) resulted in time-dependent production of nitric oxide (NO) and prostaglandin E_2 (PGE_2). This stimulation was also accompanied by the production of other reactive oxygen species such as superoxide (O_2^-), and by increased expression of COX-2. Our results provide evidence that O_2^- may be involved in the pathways that result in arachidonate release and PGE_2 formation by COX-2 in murine peritoneal macrophages stimulated by LPS. However, we were not able to demonstrate that NO participates in the regulation of PG production under our experimental conditions.

Keywords: Arachidonic acid, prostaglandins, phospholipase A_2, oxidative stress, cyclooxygenase-2

INTRODUCTION

Nitric oxide (NO) is a molecule synthesized from the amino acid L-arginine by a family of enzymes, the nitric oxide synthases (NOS). In recent years, NO has been unveiled as a potent agent in a number of important biochemical pathways. Thus, NO has been shown to function as a vasodilator, a neurotransmitter, an inhibitor of platelet aggregation, and an antimicrobial agent generated by macrophages, to name just a few of its biological roles.[1] Prostanoids, the arachidonic acid metabolites of the cyclooxygenase (COX) pathway, and NO also plays major roles in regulating inflammation, immune functions and blood vessel dilatation.[2] Both COX and NOS exist in constitutive forms (COX-1 and NOS-1 and NOS-3) which are present in many cells and are responsible for the production of prostaglandins (PGs) and NO involved in physiological functions; and in inducible forms (COX-2 and NOS-2) which are rapidly up-regulated upon appropriate stimulation.

Interactions between PGs and NO are of particular interest at sites of inflammation, where

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their synthesis is often elicited by the same stimuli,[3,4] and where they may exert synergistic as well as antagonistic actions on common targets. Recently, several conflicting reports have been published on the effect of NO on COX and/or the effect of NO on PG formation in biological systems. Thus, Kanner *et al.*[5] reported that NO was a strong inhibitor of COX. In contrast, Salvemini *et al.*[6] and Hajjar *et al.*[7] showed that NO directly stimulates the activity of purified COX, while Tsai *et al.*[8] reported that NO had a negligible effect on COX activity. At the cellular level, Hempel *et al.*[9] showed that NO synthase inhibitors did not significantly affect the formation of PGE_2 or expression of COX protein in human alveolar macrophages. However, a number of other studies report that NO stimulates PG formation in cell culture,[6,10,11] in tissue,[12,13] and in animal models.[14,15]

In this study, we investigated the potential effects of NO on several steps of the arachidonate cascade such as arachidonic acid mobilization and arachidonate metabolism by the cyclooxygenase pathway.

MATERIALS AND METHODS

Materials

Phosphatidylcholine L-α-1-palmitoyl 2-arachidonyl [arachidonyl-1-^{14}C] (60–180 Ci/mmol) and [5,6,8,9,11,12,14,15-^{3}H] Arachidonic acid ([^{3}H]AA) (180–240 Ci/mmol) were obtained from DuPont-New England Nuclear (Boston, MA). Aprotinin, leupeptin, dimethyldithiocarbamic acid, α_2-macroglobulin, phenylmethylsulfonylfluoride (PMSF), 3-morpholinosydnonimine (SIN-1), lipopolysaccharides (LPS) from *E. coli*, p-nitro blue tetrazolium (NBT), resveratrol, xanthine, xanthine oxidase and superoxide dismutase (SOD) from bovine erythrocytes were purchased from Sigma Chemical Co. (St. Louis, MO). N^{G}-methyl-L-arginine (L-NMMA) was obtained from Alexis Corp. (Coger, France). Cell culture medium RPMI 1640, fetal calf serum (FCS), penicillin G, and streptomycin were supplied by Gibco RBL (Gaithersburg, MD). COX-2 and polyclonal antiserum directed against COX-2 were from Cayman Chemicals Co. (Ann Arbor, MI). The enhanced chemiluminescence (ECL) kit was purchased from Amersham (Buckinghamshire, UK). All other reagents were of analytical grade.

Isolation and Culture of Resident Peritoneal Macrophages

Mouse peritoneal macrophages were collected from male CD-1 mice (Interfauna Iberica, Barcelona, Spain) (20–25 g), which were killed by carbon dioxide asphyxiation, and the peritoneal cavity was lavaged with PBS containing 1% BSA, 20 units of heparin/ml, 100 units of penicillin/ml, and 100 µg/ml of streptomycin. Lavage fluids were pooled and centrifuged at 400 g for 10 min at 4°C to pellet cells. Macrophages were resuspended in RPMI 1640 medium supplemented with the antibiotics and 10% FCS, counted, and plated in 12-well plastic cluster dishes (Costar, Cambridge, MA). Macrophages were allowed to adhere for 2 h at 37°C in an atmosphere of CO_2 : air (1 : 19) and 100% humidity, and the nonadherent cells were removed by washing the cell sheet in PBS.

Determination of Phospholipase A_2 Activity

Phospholipase A_2 (PLA_2) activity was determined in membrane fractions from macrophages. For this purpose, at the end of the incubation periods, the medium was replaced by cold buffer (20 mM Tris/HCl, pH 8.0, 5% saccharose) and the cells were scraped off by a rubber policeman and briefly homogenized in the cold. To prepare the membrane-fraction, the homogenate was centrifuged at 100,000*g* for 1 h. The pellet was resuspended and proteins were assayed. PLA_2 activity was measured using phosphatidylcholine L-α-1-palmitoyl 2 arachidonyl [arachidonyl-1-^{14}C] as a substrate[16] as previously described.[17]

Incorporation and Release of [^{3}H]AA

After a 20 h incubation of macrophages, the medium was removed and replaced with 0.5 ml of RPMI containing 0.1 μCi of [^{3}H]AA, and the samples were incubated for an additional 6 h at 37°C. Cells were then washed three times in Ca^{2+}- and Mg^{2+}-free PBS containing 0.5% BSA to remove unincorporated [^{3}H]AA. Macrophages used in these studies incorporated 49 ± 3% of the [^{3}H]AA under our experimental conditions. At the end of each experiment, the cell monolayer was overlaid with 1% Triton X-100, and the cells were scraped off the dishes. Finally, radioactivity present in the cell fraction was also measured by scintillation counting. The amount of [^{3}H]AA released into the medium as a result of the treatment is expressed as a percentage of cell-incorporated [^{3}H]AA, which was determined in solubilized cells. Background [^{3}H]AA release from untreated cells was subtracted from all data.

Nitrite Assay

Nitrite, a stable end product of NO, was measured in the culture media by using the spectrophotometric Greiss reaction.[18] An aliquot of sample (180 μl) was mixed with 40 μl of Greiss reagent (1% sulfanylamide and 0.1% naphthylethylenediamine dihydrochloride in 2% phosphoric acid). The mixture was incubated for 10 min at room temperature, and the absorbance at 550 nm was measured. Nitrite concentrations were determined by comparison with a sodium nitrite standard curve. The lower limit of detection was 0.2 μmol/l.

Superoxide Assay

Superoxide anion (O_2^-) concentrations were determined by measuring the SOD inhibitable reduction of blue tetrazolium.[19] Thus, the reduction of NBT to formazan was assessed by measurement of optical density at 560 nm. A flux of O_2^- was used as standard curve. This O_2^- flux was generated during the oxidation of xanthine (100 μM) to urate by xanthine oxidase (5 mM), with standard conditions set such that a solution of 10 μM cytochrome c was reduced at a rate of 0.025 ± 0.001 absorbance units/min at 550 nm.[20]

Measurement of PGE_2 Levels

An aliquot of culture supernatant medium (0.25 ml) was acidified with 1 ml of 1% formic acid. PGE_2 was extracted in ethyl acetate (5 ml), and, after the aqueous phase was discarded, the organic phase was evaporated in a stream of nitrogen. The overall recovery for the extraction procedures was established by including [^{3}H]-PGE_2 and was found to be 82%. PGE_2 levels in the medium were determined using a PGE_2-monoclonal enzyme immunoassay kit (Cayman Chemicals Co.), following the manufacturer's protocol.

Protein Determination

Total protein was measured by the Bradford method[21] by means of the Bio-Rad protein assay, using BSA as the standard.

Western Blot Analysis of COX-2

Cells were washed twice with ice-cold PBS, scraped off in PBS containing 2 mM EDTA and pelleted. Cell pellets were sonicated in PBS containing 2 mM EDTA, 2 μg/ml PMSF, 20 μg/ml aprotinin, 20 μg/ml leupeptin, 200 μg/ml dimethyldithiocarbamic acid, and 0.2 mg/ml α_2-macroglobulin. Immunoblot analysis for COX-2 was performed as follows: cell lysates with equal amounts of protein (20 μg) were separated by a 10% SDS-PAGE gel[22] and blotted for 1 h with a constant current of 250 mA onto a nitrocellulose membrane (Trans-blot Bio-Rad, 0.4 μm pore size) using a MiniProtean II system (Bio-Rad, Hercules, CA). Sheep COX-2 purified from placenta were also loaded on the gels as a positive control. The membranes were blocked with 5% nonfat milk

powder in PBS–0.1% Tween 20 for 1 h. A rabbit polyclonal antiserum directed against COX-2 was applied in a dilution of 1 : 2000 for 1 h. These anti-COX-2 did not cross-react with COX-1.[23] The blot was washed several times with PBS–Tween 20 and incubated with a goat anti-rabbit antibody in a 1 : 2000 dilution for 1 h. Antibody binding was visualized by the ECL technique according to the instructions of the supplier, using Kodak X-OMAT LS film. Quantitation was by videodensitometry.

Statistical Analysis

For the cell culture experiments, data are expressed as the mean ± standard error of the mean (SE). All experiments were performed at least three times to ensure consistency of the observations. The significance of differences between data points and the control was determined using a two-tailed Student's *t*-test with $P < 0.05$ confidence limits.

RESULTS

Time-Dependent Production of Mediators and Related Enzymes after Stimulation of Macrophages by LPS

LPS induced significant early O_2^- and PGE_2 production. O_2^- levels reached a plateau after 2 h, whereas PGE_2 production continued to increase, up to 24 h (Table I). On the other hand, nitrite, the stable reaction product of NO with molecular oxygen, suffered an appreciable enhancement in the supernatant 18 h after LPS activation, and increased up to 24 h (Table I).

Lysates from the same cells were subjected to SDS-PAGE, and Western blot analysis was performed using a specific anti-COX-2 antibody. Low immunoreactivity was observed in unstimulated cells. However, when we incubated macrophages with LPS (1 μg/ml) an increase in COX-2 protein levels was observed 2 h after LPS stimulation (Figure 1A). COX-2 levels were high between 2 and 12 h after LPS exposure and subsequently decreased. Thus, our results show marked differences in the time-course of O_2^-

TABLE I LPS induces a time-dependent accumulation of nitrite, O_2^- and PGE_2 in mouse peritoneal macrophages

	Nitrite (nmol/ml)	O_2^- (nmol/ml/20 min)	PGE_2 (pg/ml)
Control	2.0 ± 0.1	4.7 ± 0.6	25 ± 2
LPS (2 h)	2.5 ± 0.1	25.6 ± 4.6[a]	263 ± 5[a]
LPS (6 h)	3.1 ± 0.3	27.3 ± 6.5[a]	423 ± 12[a]
LPS (6 h) + resveratrol	ND	16.7 ± 1.9[b]	289 ± 11[b]
LPS (18 h)	37.5 ± 1.1[a]	24.2 ± 3.5[a]	1056 ± 21[a]
LPS (24 h)	43.0 ± 1.2[a]	18.3 ± 2.7[a]	1763 ± 27[a]
LPS (24 h) + L-NMMA	12.1 ± 0.8[c]	17.9 ± 2.1	1785 ± 23[a]

Isolated macrophages (10^6) were incubated in serum-free RPMI 1640 medium (1 ml) in the presence or absence of LPS (1 μg/ml). L-NMMA and resveratrol were used at 1 mM and 50 μM, respectively. At the indicated times, aliquots of the medium were removed and its nitrite, O_2^- or PGE_2 content were analyzed, as indicated in the Materials and Methods. Results are the mean ± SE of three individual experiments containing three replicates per condition.
[a]Significantly different from control values, [b]different from LPS (6 h) and [c]different from LPS (24 h) values by Student's *t*-test ($P < 0.05$) (ND, not determined).

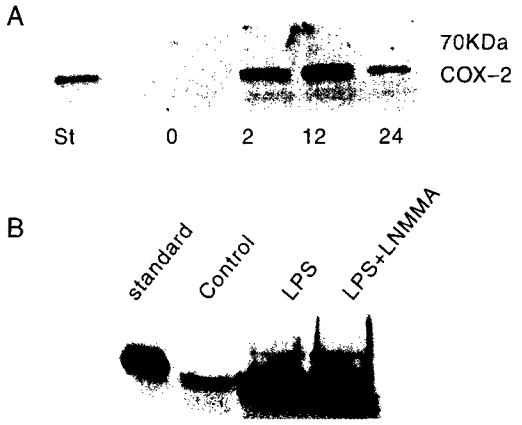

FIGURE 1 (A) Time-dependent expression of COX-2 in mouse macrophages stimulated with LPS (1 μg/ml). Stimulation was stopped by removal of supernatants, then macrophages were lysed and proteins (20 μg) were subjected to SDS-PAGE. Ovine COX-2 (100 ng) was used as standard (St). COX-2 immunodetection was performed as described in the Materials and Methods. (B) Effect of L-NMMA (1 mM) on COX-2 overexpression induced by LPS (1 μg/ml) for 12 h. Results are representative of three separate experiments.

TABLE II Effect of LPS on PLA_2 activity and [^{3}H]AA released in murine peritoneal macrophages

	PLA_2 activity	% [^{3}H]AA released
Control	39 ± 3	
LPS (2 h)	246 ± 8^a	32.8 ± 1.9^a
LPS (6 h)	265 ± 11^a	45.7 ± 1.7^a
LPS (18 h)	ND	48.9 ± 2.1^a
LPS (24 h)	224 ± 7^a	51.2 ± 1.9^a
LPS (24 h) + L-NMMA	221 ± 9^a	49.8 ± 2.1^a

Macrophages incubated in LPS (1 μg/ml) were homogenated and centrifuged (see Materials and Methods) to separate the membrane fraction. PLA_2 activity was measured in the pellet fraction and was expressed as pmol of arachidonic acid hydrolyzed/min/mg protein. [^{3}H]AA prelabeled macrophages were used to determine the percentage of [^{3}H]AA release by LPS (1 μg/ml). The background of [^{3}H]AA released by control cells at the indicated times ($9 \pm 1\%$ to $15 \pm 2\%$) was subtracted from all data. L-NMMA was used at 1 mM. Results are the mean ± SE of three individual experiments containing three replicates per condition. [a]Significantly different from control values by Student's *t*-test ($P < 0.05$) (ND, not determined).

release, COX-2 induction, PGE_2 synthesis and nitrite production induced by LPS.

L-NMMA, a competitive inhibitor of NO synthesis, inhibited the production of nitrite without modifying significantly PGE_2 formation stimulated by LPS in our experimental conditions. However, resveratrol, an natural antioxidant that acts as scavanger of O_2^- [24] reduced PGE_2 levels increased by LPS (Table I). Moreover, Western blot analysis of the cell lysates incubated under these conditions showed similar COX-2 expression in the presence of L-NMMA compared with LPS alone (Figure 1B). In the absence of LPS, L-NMMA did not modify PGE_2 levels and there was also no variation in COX protein levels (data not shown).

As we can see in Table II, LPS-induced [^{3}H]AA mobilization in [^{3}H]AA prelabeled cells during the period studied and these data can be correlated with a significant enhancement of PLA_2 activity in the membrane fraction in LPS-stimulated macrophages (Table II). However, the addition of L-NMMA did not modify the effect of LPS on PLA_2 activity in the membrane fraction and the subsequent [^{3}H]AA release (Table II).

NO and O_2^- Generation by SIN-1

Noack *et al.*[25] reported that the anti-anginal effect of molsidomine is due to its hepatic metabolite, SIN-1, which spontaneously decomposes by releasing NO. However, we must consider that SIN-1 produces NO and superoxide anions,[26,27] which react to form peroxynitrite.

Because the chemistry of SIN-1 in culture medium is complex and not understood, the SIN-1 treatment of the cells was conducted in a simple solution (PBS solution). In order to produce a flux of NO in the presence or absence of O_2^-, SIN-1/SOD were used. Our results show that SIN-1 (0.1–1 mM) was able to produce nitrite and O_2^- at concentrations similar to that detected in macrophage cultures stimulated by LPS (1 μg/ml). Addition of SOD (25 units/ml) to the medium markedly reduced O_2^- produced by SIN-1 without decreasing nitrite concentrations (Table III). The effect of SIN-1 on macrophage viability was also evaluated (data not shown), and noncytotoxic concentrations (0.1–1 mM) were then chosen to continue the study.

Effect of SIN-1 on PLA_2 Activity, AA Mobilization, COX-2 Expression and Prostanoid Biosynthesis in Peritoneal Macrophages

The production of NO/O_2^- by SIN-1 induced a significant increase in PLA_2 activity in the membrane fraction of macrophages (Figure 2) and the subsequent [^{3}H]AA release that was significant when nitrite/O_2^- concentrations in the culture medium were similar to the concentrations released during LPS (1 μg/ml) exposure (Figure 3). These experiments were also performed in the presence of SOD (25 units/ml) to neutralize O_2^-. In such conditions, the enhancement of PLA_2 activity and [^{3}H]AA release were markedly reduced (Figure 3).

We also determined the effect of exposure of macrophages to O_2^- and/or NO produced by SIN-1 in the absence or presence of SOD, on

TABLE III Nitrite and O_2^- produced by SIN-1

	Nitrite (nmol/ml)		O_2^- (nmol/ml/20 min)	
	30 min	120 min	30 min	120 min
Control	2.03 ± 0.1	2.05 ± 0.1	6.5 ± 0.8	6.5 ± 1.4
SIN-1 (0.1 mM)	12.56 ± 0.75[a]	19.23 ± 0.65[a]	23.3 ± 2.8[a]	28.9 ± 4.7[a]
SIN-1 (0.1 mM) + SOD	11.99 ± 0.61[a]	18.76 ± 0.55[a]	8.4 ± 1.6[b]	9.3 ± 0.4[b]
SIN-1 (1 mM)	38.33 ± 1.22[a]	47.93 ± 3.51[a]	33.6 ± 5.7[a]	38.3 ± 4.8[a]
SIN-1 (1 mM) + SOD	39.64 ± 2.01[a]	51.32 ± 2.91[a]	10.3 ± 1.5[b]	10.2 ± 2.2[b]

Nitrite and O_2^- produced by SIN-1 in the presence or absence of SOD (50 units/ml) were measured as described in the Materials and Methods. Results are the mean ± SE of four individual experiments containing two replicates per condition. [a]Significantly different from control values and [b]different from cells stimulated with SIN-1 (Student's *t*-test, $P < 0.05$).

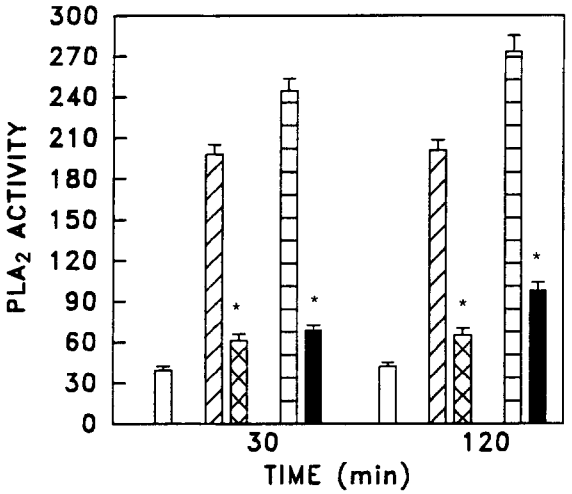

FIGURE 2 Effect of SIN-1 exposure on PLA_2 activity (pmol AA hydrolyzed/min/mg protein) in the membrane fraction. Resident peritoneal macrophages were incubated for 30 and 120 min at 37°C with SIN-1 solution in PBS (0.1 mM, ▨▩ or 1 mM, ▤■) in the presence (▩■) or absence (▨▤) of SOD (25 units/ml) to expose cells to different NO concentrations in the presence or absence of O_2^- (control values □). Results are the mean ± SE of four individual experiments each containing two replicates. *Significantly different from SIN-1 in absence of SOD (Student's *t*-test, $P < 0.01$).

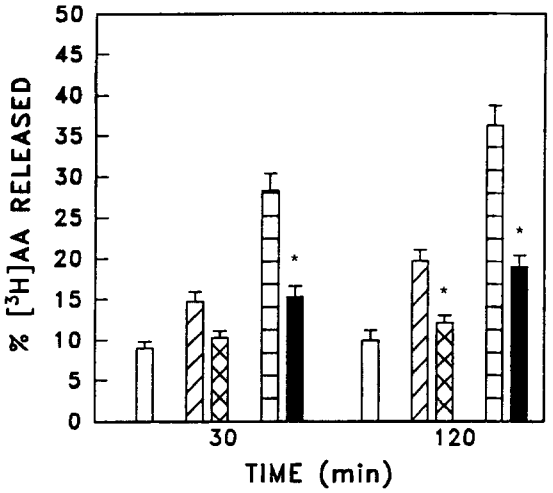

FIGURE 3 Effect of SIN-1 exposure on [^{3}H]AA release. Resident peritoneal macrophages prelabeled with [^{3}H]AA were incubated for 30 and 120 min at 37°C with SIN-1 solution in PBS (0.1 mM, ▨▩ or 1 mM, ▤■) in the presence (▩■) or absence (▨▤) of SOD (25 units/ml) to expose cells to different NO concentrations in the presence or absence of superoxide radicals. (Control values □). Results are the mean ± SE of four individual experiments each containing two replicates. *Significantly different from SIN-1 in the absence of SOD (Student's *t*-test, $P < 0.01$).

cellular COX-2 protein levels. SIN-1 (0.1 mM) was not able to induce obvious COX-2 overexpression (data not shown); although we observed an increase in PGE_2 formation after SIN-1 exposure (Figure 4). However, this effect was significantly inhibited when we added SOD to the medium to neutralize O_2^- production. Isoprostanes are PG-like compounds that are produced independently of the COX enzyme by free radical catalyzed peroxidation of AA.[28] Thus, Moore *et al.*[29] demonstrated that peroxynitrite and SIN-1 can induce the formation of F2-isoprostanes in lipoproteins. We used ketoprofen, an non-steroidal anti-inflammatory drug and COX inhibitor[30,31] to demonstrate that ROS produced by SIN-1 induced PGE_2 production by COX pathway. Thus, ketoprofen (5 μM) inhibited almost completely PGE_2 synthesis stimulated by SIN-1 (Table IV).

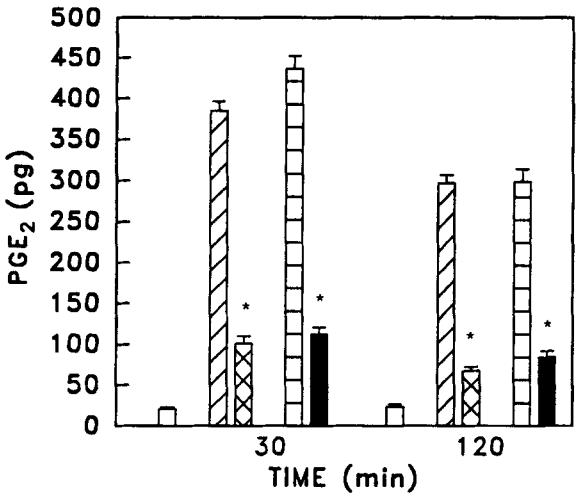

FIGURE 4 Effect of SIN-1 exposure on PGE_2 release. Resting peritoneal macrophages were incubated for 30 and 120 min at 37°C with SIN-1 solution in PBS (0.1 mM, ▨⊠ or 1 mM, ⊟■) in the presence (⊠■) or absence (▨⊟) of SOD (25 units/ml) to exposure cells to different NO concentrations in the presence or absence of O_2^-. Results are the mean ± SE of four individual experiments each containing two replicates. *Significantly different from SIN-1 in the absence of SOD (Student *t*-test, $P < 0.01$).

TABLE IV Effect of ketoprofen on PGE_2 produced by SIN-1

	PGE_2 (pg/ml)
Control	18 ± 1
SIN-1 (0.1 mM)	286 ± 8
SIN-1 (0.1 mM) + ketoprofen	49 ± 3
SIN-1 (1 mM)	301 ± 11
SIN-1 (1 mM) + ketoprofen	75 ± 5

Resting peritoneal macrophages were incubated for 120 min at 37°C with SIN-1 solution in PBS (0.1 and 1 mM) in the presence or absence of ketoprofen (5 μM). Results are the mean ± SE of three individual experiments each containing two replicates.

DISCUSSION

In macrophages the respiratory burst oxidase is normally inactive but it can be stimulated by different ligands such as opsonized particles, microorganisms or LPS. This releases reactive oxygen species such as O_2^- and hydrogen peroxide which are involved in the pathways that result in arachidonate release, a limiting step in the biosynthesis of prostanoids.[32] Free radical NO has emerged as another important molecule implicated in inflammation,[33] and increasing evidence indicates that NO can stimulate or inhibit PG synthesis, depending on the cell type under investigation. Thus, conflicting reports have been published on the interaction of NO and the COX pathway and subsequent PG formation.[5–8] Moreover, NO couples to O_2^- at a diffusion-controlled rate to produce peroxynitrite.[27] The occurrence of this reaction in macrophages contributes to their cytotoxic activity towards invading pathogens, because peroxynitrite and its conjugate acid, peroxynitrous acid, are potent oxidizing agents.[34,35] However, because peroxynitrite is an inorganic hydroperoxide, it is also a potential substrate for the peroxidase activity of COX and an activator of the enzymatic activity.[18]

Our results show that murine peritoneal macrophages stimulated by LPS increased the production of reactive oxygen species such as O_2^- and NO and that these effects were accompanied by an increased biosynthesis of PGE_2. Interestingly these events occurred with a different time-course. Thus, O_2^- and PGE_2 production were more early events than NO release.

We also investigated the capacity of NO and O_2^- to control PGE_2 formation by modulating the corresponding enzymes of the arachidonate cascade such as PLA_2 and COX-2. Oxygen species have been shown to trigger AA release.[32] A role for cytosolic PLA_2 has often been suggested but not clearly demonstrated. Our findings indicate that the production of O_2^- and PGE_2 by macrophages in response to LPS stimulation was accompanied by a parallel rise in the activity of the corresponding enzymes. Thus, we observed a rapid increase in PLA_2 activity in the membrane fraction. As previously reported, this PLA_2 translocation to the membrane fraction may be correlated with the enhancement of [^{3}H]AA mobilization.[32] Thus, free-arachidonic acid is the substrate used by COX to produce PGs. The time-dependent increase in the expression of the COX-2 protein induced by LPS suggests

a role for this inducible isoform together with arachidonate release in PGE_2 formation caused by LPS. It is important to consider that all of these events were observed 2 h after LPS stimulation when we did not notice a significant nitrite production. Thus, NO produced by LPS-stimulated macrophages may not be responsible for AA mobilization and/or COX-2 induction, since these events precede NO production. Moreover, the abrogation of endogenous NO synthesis by the addition of the NOS inhibitor, L-NMMA, did not result in any apparent change in LPS-induced PLA_2 translocation, [^{3}H]AA mobilization, COX-2 expression and the subsequent PGE_2 release.

These results also suggest that NO did not participate in the control of PGE_2 formation induced by LPS in our experimental conditions. In contrast, we must consider that O_2^- may be implicated in the mechanism that leads to the initial LPS-induced PGE_2 release.

Many transcription factors are regulated by oxygen radicals.[36] Thus, we can hypothesize that, as an oxidizing radical, NO and/or O_2^- could activate an early-immediate gene leading to new synthesis of COX-2. Stimulatory as well as inhibitory interactions between the NO and COX pathway have been documented. Thus, the inhibitory activity of NO was reported in the J744.2 macrophage cell line[37] whereas stimulatory activity has been reported by Salvemini *et al.*[6] using the mouse macrophage cell line RAW 264.7. To confirm whether NO and/or O_2^- are elements of signal pathways involved in prostanoid production by murine peritoneal macrophages, we determined the effect of NO and/or O_2^- exposure on PGE_2 biosynthesis. For this purpose, we used SIN-1, a NO/O_2^- donor, to reproduce the NO and O_2^- concentrations that we detected in cell cultures stimulated by LPS. We observed that SIN-1 induced an increase in PLA_2 activity in the membrane fraction and subsequent [^{3}H]AA mobilization. However, all of these events were markedly inhibited in the presence of SOD. Thus, these findings demonstrate that the above biological effects induced by SIN-1 following reactive oxygen species exposure such as O_2^- could be independent of the presence of NO and/or peroxynitrite.

In conclusion, our results provide evidence that O_2^- could be involved in the pathways that result in AA mobilization and PGE_2 release by COX-2 stimulated by LPS; whereas there was no evidence that NO participates in the regulation of PGE_2 production in our experimental conditions.

Acknowledgments

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