ELSEVIER

European Journal of Pharmacology 286 (1995) 219–222

ejp

Short communication

Endogenous nitric oxide increases prostaglandin biosynthesis in carrageenin rat paw oedema

Lidia Sautebin *, Armando Ialenti, Angela Ianaro, Massimo Di Rosa

Department of Experimental Pharmacology, University of Naples 'Federico II', Via Domenico Montesano, 49, 80131 Naples, Italy

Received 12 September 1995; accepted 14 September 1995

Abstract

The influence of endogenous nitric oxide (NO) on prostaglandin biosynthesis in rat carrageenin oedema was studied. Oedema formation and prostaglandin E_2 generation in the inflamed paw were both increased by L- but not D-arginine and reduced by the NO synthase inhibitor, L-N^G-nitro arginine methyl ester, and the NO scavenger, haemoglobin. Methylene blue, an inhibitor of the soluble guanylate cyclase, had no effect. These results suggest that endogenous NO modulates carrageenin oedema by increasing prostaglandin biosynthesis at the inflammatory site through a cGMP-independent mechanism.

Keywords: Carrageenin edema; Nitric oxide (NO); Prostaglandin

1. Introduction

Nitric oxide (NO) is formed from L-arginine by the enzymatic action of NO synthase and acts as the endogenous stimulator of the soluble guanylate cyclase (Moncada et al., 1991).

Rat paw oedema induced by carrageenin is characterized by an early phase (1–2 h) brought about by the release of histamine, 5-hydroxytryptamine and bradykinin followed by a late phase (3–4 h) mainly sustained by prostaglandin release (Di Rosa et al., 1971; Di Rosa and Willoughby, 1971). Endogenous NO is also involved in this type of acute inflammation since oedema formation is increased by L-arginine and decreased by NO synthase inhibitors (Ialenti et al., 1992). We have recently shown that prostacyclin biosynthesis in the lung of endotoxin-treated rats is increased by L-arginine and reduced by NO synthase inhibitors, suggesting that endogenous NO may modulate prostanoid production (Sautebin and Di Rosa, 1994). Furthermore we have demonstrated that NO increases arachidonic acid-stimulated prostaglandin biosynthesis in vivo by activating cyclooxygenase through a cGMP-independent mechanism (Sautebin et al., 1995). These results suggest that endogenous NO may play a relevant role in the modulation of prostaglandin generation at the inflammation site.

In this study we investigated the influence of endogenous NO on prostaglandin biosynthesis in rat carrageenin oedema. We measured both the foot volume and the amounts of prostglandin E_2 in the oedematous fluid recovered from inflamed paws.

2. Materials and methods

Paw oedema was induced in male Wistar rats (Nossan, Italy; 140–160 g) by subplantar injection of 0.1 ml saline containing 1% λ-carrageenin in the presence or absence of L-arginine, D-arginine, L-N^G-nitro arginine methyl ester, haemoglobin, methylene blue and indomethacin (all from Sigma). The volume of the paw was measured with a plethysmometer (Basile, Italy) immediately after the injection as previously described (Di Rosa and Willoughby, 1971). Subsequents readings of the same paw were carried out at 1-h intervals up to 5 h and compared to the initial readings. The increase in paw volume was taken as oedema volume. In some experiments the rats were killed in an atmosphere of CO_2 immediately after the readings at 3 h. Each inflamed paw was cut at the level of the calcaneus using a guillotine and gently spun ($250 \times g$ for 15 min) in

* Corresponding author. Tel.: +39-81-7486427; fax: +39-81-7486403.

Elsevier Science B.V.
SSDI 0014-2999(95)00581-1

order to recover a sample of the oedematous fluid. The blood was removed by filtering the samples through a 10000 M_r cut filter (Centricon 10, Amicon). The amount of prostaglandin E_2 in samples of oedematous fluid was measured by radioimmunoassay (Ciabattoni et al., 1979). The total amount (TA) of prostaglandin E_2 (PGE_2) present in the entire oedematous fluid of each paw was calculated as follows:

$$TA = \frac{(PGE_2 \text{ in the sample (pmol)}) \times (\text{paw oedema volume (ml)})}{(\text{sample volume (ml)})}$$

The data are expressed as means ± S.E.M. Comparisons were made by means of the unpaired two-tailed Student's t-test. The level of a statistically significant difference was defined as $P < 0.05$.

3. Results

In preliminary experiments we established that injection into the rat paw of L-N^G-nitro arginine methyl ester (0.1 μmol), haemoglobin (30 μmol), methylene blue (3 μmol), L- or D-arginine (15 μmol) did not produce any detectable oedema. Carrageenin oedema was greatly reduced by coinjection of 3 μmol indomethacin, 0.1 μmol L-N^G-nitro arginine methyl ester, 30 μmol haemoglobin whereas it was unaffected by 3 μmol methylene blue (Fig. 1). Coinjection of L- but not D-arginine (15 μmol) significantly enhanced, by about 50%, the carrageenin-induced oedema. Indomethacin (3 μmol), L-N^G-nitro arginine methyl ester (0.1 μmol) and haemoglobin (30 μmol) produced a marked inhibition of L-arginine + carrageenin oedema whereas oedema was unaffected by 3 μmol methylene blue (not shown).

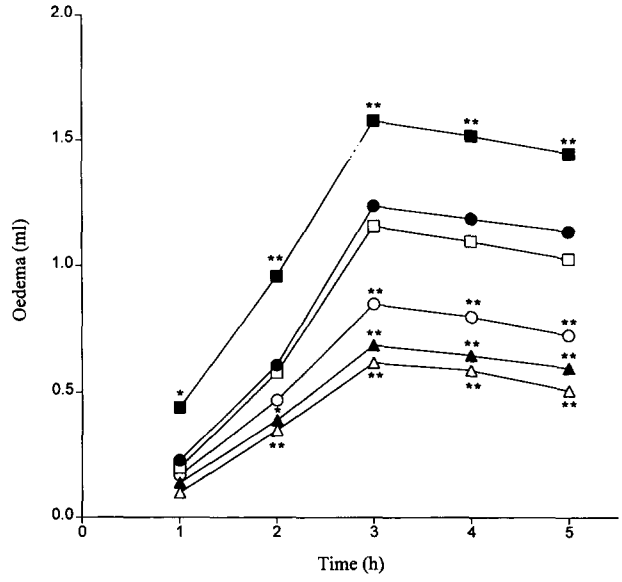

Fig. 1. Effect of 0.1 μmol L-N^G-nitro arginine methyl ester (△), 3 μmol indomethacin (○), 30 μmol haemoglobin (▲), 3 μmol methylene blue (□), 15 μmol L-arginine (■) on rat carrageenin oedema. The oedema induced by carrageenin alone (control) is shown by solid circles (●). Each point represents the mean for 5–6 rats. Standard errors were always less than 10% of the respective means and are not shown. $^*P < 0.05$, $^{**}P < 0.01$ vs. control group.

The amounts of prostaglandin E_2 found in the oedematous fluid recovered from the inflamed paws correlated well with the severity of the oedema induced by carrageenin alone or in combination with the various agents (Table 1).

Table 1
Alteration in oedema formation and amounts of prostaglandin E_2 in rat paw oedema induced by 0.1 ml 1% λ-carrageenin given alone (group 1, control) or coinjected with various agents

Group	Agents (μmol per paw)	Oedema (ml at 3 h)	PGE_2 (pmol per paw)	n
1	Control	1.10 ± 0.07	301 ± 11	10
2	Indomethacin 3	0.76 ± 0.04 [a]	174 ± 10 [b]	4
3	L-NAME 0.1	0.56 ± 0.04 [a]	141 ± 12 [b]	4
4	Hb 30	0.65 ± 0.06 [a]	181 ± 10 [b]	4
5	Mb 3	1.04 ± 0.03	318 ± 15	4
6	L-Arginine 15	1.57 ± 0.11 [b]	425 ± 10 [b]	8
7	D-Arginine 15	1.03 ± 0.04	315 ± 30	4
8	L-Arginine 15 + indomethacin 3	0.70 ± 0.06 [d]	187 ± 21 [d]	4
9	L-Arginine 15 + L-NAME 0.1	1.00 ± 0.14 [c]	171 ± 18 [d]	4
10	L-Arginine 15 + Hb 30	1.13 ± 0.06 [c]	190 ± 17 [d]	4
11	L-Arginine 15 + Mb 3	1.62 ± 0.05	443 ± 14	4

The amounts of prostaglandin E_2 (PGE_2) were measured by radioimmunoassay in samples of the oedematous fluid recovered from the inflamed paws 3 h after the injections and are expressed as total amount present in each paw. L-N^G-Nitro arginine methyl ester (L-NAME); haemoglobin (Hb); methylene blue (Mb). [a] $P < 0.05$, [b] $P < 0.01$ vs. group 1; [c] $P < 0.05$, [d] $P < 0.001$ vs. group 6.

4. Discussion

In this study we have shown that endogenous NO increases prostaglandin E_2 biosynthesis and enhances oedema formation in the rat paw injected with carrageenin. Thus oedema was significantly reduced, not only by the cyclooxygenase inhibitor, indomethacin, but also by the NO synthase inhibitor, L-N^G-nitro arginine methyl ester, and the NO scavenger, haemoglobin. The inhibition of carrageenin-induced oedema by L-N^G-nitro arginine methyl ester (0.1 μmol/paw) was in agreement with our previous results (Ialenti et al., 1992). Furthermore it has been shown that both bradykinin- and 5-hydroxytryptamine-induced rat paw oedema were reduced by 0.15 μmol/paw of this NO synthase inhibitor whereas these inflammatory responses were potentiated when greater amounts of the inhibitor (15 μmol/paw) were used (Giraldelo et al., 1994). The potentiation of paw oedema was apparently dependent on histamine release caused by mast cell degranulation due to the cationic charge of the compound. The release of histamine could explain the reported ability of L-N^G-nitro arginine methyl ester (2 mg/kg i.v.) to increase both basal and PAF-induced albumin extravasation in several organs of the rat except skin (Filep and Foldes-Filep, 1993). Indeed we found that L-N^G-nitro arginine methyl ester (0.1 μmol/site) had no effect on basal vascular permeability of rat skin but reduced by 30–40% the carrageenin-induced plasma extravasation (Ialenti et al., 1992). Both L-N^G-nitro arginine methyl ester and haemoglobin also produced a concomitant marked reduction of the amounts of prostaglandin E_2 in the oedematous fluid recovered from inflamed paws. We were not able to measure the nitrite/nitrate content in oedematous fluid since, with the amounts recovered, the samples were just enough for the prostaglandin E_2 radioimmunoassay. However, in a model of carrageenin pleurisy with rats treated with the same agents as used in the present study, by measuring both nitrite/nitrate content and prostaglandin E_2 level in individual samples of pleural exudate, we found that the inhibition of endogenous NO paralleled the reduction of prostaglandin E_2 (unpublished observations).

L-Arginine, the substrate for NO generation, greatly enhanced carrageenin oedema and produced a well correlated increase of prostaglandin E_2 amounts in the oedematous fluid, whereas D-arginine had no effect. Both oedema formation and prostaglandin biosynthesis induced by coinjection of carrageenin and L-arginine were concomitantly reduced by L-N^G-nitro arginine methyl ester, haemoglobin and indomethacin. The inhibitor of soluble guanylate cyclase, methylene blue, was unable to modify either the oedema or the prostaglandin E_2 biosynthesis induced by carrageenin or carrageenin + L-arginine. It has been reported that methylene blue, although considered as an inhibitor of soluble guanylate cyclase, inhibits purified brain NO synthase and can trap free NO (Mayer et al., 1993). These mechanisms do not seem to play a role in carrageenin inflammation since the inhibition of NO synthase with L-N^G-nitro arginine methyl ester and the scavenging of NO with haemoglobin both resulted in a reduction of oedema volume and prostaglandin E_2 formation. Interestingly the dose of methylene blue we used was highly effective to reduce the oedema induced by the NO donor, 3-morpholino-sydnonimine-hydrochloride (SIN-1), which acts through a cGMP-dependent mechanism (Sautebin et al., 1995).

The results of this study suggest strongly that endogenous NO is formed at the inflammation site and contributes to oedema formation by increasing prostaglandin biosynthesis through a cGMP-independent mechanism which possibly depends on a direct interaction of NO with the iron-heme centre of cyclooxygenase. This view is supported by recent observations showing that endogenous NO activates cyclooxygenase and enhances prostaglandin biosynthesis either in vitro (Salvemini et al., 1994) or in vivo (Sautebin et al., 1995).

In carrageenin inflammation NO may be generated either by the vascular endothelial cells, via the constitutive NO synthase stimulated by some mediators released in the early phase of the oedema (histamine, 5-hydroxytryptamine, bradykinin) or by the inducible enzyme carried to the inflammation site by the infiltration of leucocytes activated by the phlogogenic agent (Sturm et al., 1989). Carrageenin oedema is characterized by massive infiltration of inflammatory cells which are a major source of prostaglandins at the inflammation site (Di Rosa et al., 1971; Di Rosa and Willoughby, 1971). Therefore the possibility that NO might increase prostaglandin biosynthesis by promoting leucocyte migration at the inflammation site should be considered, although this mechanism appears rather unlikely since several lines of evidence implicate NO as an endogenous inhibitor of leucocyte adherence and emigration (Kubes et al., 1991, 1993; Arndt et al., 1993). However, the relevance of these pathways for NO-driven prostaglandin biosynthesis in carrageenin oedema requires elucidation.

In conclusion, the present results suggest that the interaction between NO synthase and cyclooxygenase pathways may represent an important mechanism for modulation of the inflammatory response.

References

Arndt, H., C.W. Smith and D.N. Granger, 1993, Leukocyte-endothelial cell adhesion in spontaneously hypertensive and normal rats, Hypertension 21, 667.

Ciabattoni, G., F. Pugliese, M. Spaldi, G.A. Cinotti and C. Patrono, 1979, Radioimmunoassay mesurement of prostaglandins E_2 and $F_{2\alpha}$ in human urine, J. Endocrinol. Invest. 2, 173.

Di Rosa, M. and D.A. Willoughby, 1971, Screens for anti-inflammatory drugs, J. Pharm. Pharmacol. 23, 297.

Di Rosa, M., J.P. Giroud and D.A. Willoughby, 1971, Studies of mediators of the acute inflammatory response induced in rats in different sites by carrageenin and turpentine, J. Pathol. 104, 15.

Filep, J.G. and E. Foldes-Filep, 1993, Modulation by nitric oxide of platelet-activating factor-induced albumin extravasation in the conscious rat, Br. J. Pharmacol. 110, 1347.

Giraldelo, C.M.M., A. Zappelliti, M.N. Muscarà, I.M.S. De Luca, S. Hyslop, G. Cirino, R. Zatz, G. De Nucci and E. Antunes, 1994, Effect of arginine analogues on rat hind paw oedema and mast cell activation in vitro, Eur. J. Pharmacol. 257, 87.

Ialenti, A., A. Ianaro, S. Moncada and M. Di Rosa, 1992, Modulation of acute inflammation by endogenous nitric oxide, Eur. J. Pharmacol. 211, 177.

Kubes, P., M. Suzuki and D.N. Granger, 1991, Nitric oxide: an endogenous modulator of leukocyte adhesion, Proc. Natl. Acad. Sci. USA 88, 4651.

Kubes, P., S. Kanwar, X.F. Niu and J. Gaboury, 1993, Nitric oxide synthesis inhibition induces leukocyte adhesion via superoxide and mast cells, FASEB J. 7, 1293.

Mayer, B., F. Brunner and K. Schmidt, 1993, Inhibition of nitric oxide synthesis by methylene blue, Biochem. Pharmacol. 45, 367.

Moncada, S., R.M.J. Palmer and E.A. Higgs, 1991, Nitric oxide: physiology, pathophysiology and pharmacology, Pharmacol. Rev. 43, 109.

Salvemini, D., K. Seibert, L. Masferrer, T.P. Mislo, M.G. Currie and P. Needleman, 1994, Endogenous nitric oxide enhances prostaglandin production in a model of renal inflammation, J. Clin. Invest. 93, 1940.

Sautebin, L. and M. Di Rosa, 1994, Nitric oxide modulates prostacyclin biosynthesis in the lung of endotoxin treated rats, Eur. J. Pharmacol. 262, 193.

Sautebin, L., A. Ialenti, A. Ianaro and M. Di Rosa, 1995, Nitric oxide modulates prostaglandin biosynthesis in the rat, Br. J. Pharmacol. 114, 323.

Sturm, R.J., D.A. Holloway, S. Buckley, M.C. Osborne, D. Grimes, B.M. Weichman and T.J. Rimele, 1989, Potential regulatory role of inflammatory cells on local vascular smooth muscle tone, Agents Actions 27, 414.