Vol. 194, No. 1, 1993 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 15, 1993 Pages 420-424

NITRIC OXIDE MEDIATES INTERLEUKIN-1-INDUCED PROSTAGLANDIN E_2 PRODUCTION BY VASCULAR SMOOTH MUSCLE CELLS

Takuo Inoue, Keisuke Fukuo[1], Shigeto Morimoto, Eio Koh, and Toshio Ogihara

Department of Geriatric Medicine, Osaka University Medical School, 2-2, Yamadaoka, Suita, Osaka, 565, Japan

Received June 1, 1993

SUMMARY: We examined the possible participation of nitric oxide (NO) in the activation of cyclooxygenase, a heme-containing enzyme, induced by interleukin-1 (IL-1) in vascular smooth muscle cells (VSMC). IL-1 induced a delayed and prolonged release of both NO and prostaglandin E_2 (PGE_2) from VSMC. N^G-monomethyl-L-arginine (L-NMMA), an inhibitor of NO synthesis, partially but significantly inhibited the PGE_2 release induced by IL-1, whereas it completely inhibited the IL-1-induced NO production. The inhibitory effects of L-NMMA on IL-1-induced production of both NO and PGE_2 were partially reversed by a 10-fold excess of L-arginine. In addition, coincubation with superoxide dismutase enhanced the IL-1-induced PGE_2 release from VSMC. In contrast, 8-bromo-cyclic GMP did not stimulate PGE_2 release. Furthermore, 0.2 mM sodium nitroprusside directly stimulated PGE_2 release from VSMC. These findings suggest that NO at least in part mediates the IL-1-induced PGE_2 production, and that NO may be one of the important signals for the activation of cyclooxygenase to produce PGE_2 in VSMC.

Nitric oxide (NO) is an unstable but multifunctional molecule which mediates a number of diverse physiological processes, including smooth muscle relaxation, platelet inhibition, neurotransmission, immune regulation, and penile erection (1,2). NO is synthesized from L-arginine by NO synthase. Distinct forms of NO synthase have been molecularly cloned from brain (3), macrophages (4), and endothelium (5). NO binds with very high affinity to iron in the heme of soluble guanylate cyclase, eliciting a conformational change that enhances the catalytic activity of the enzyme to stimulate the formation of cyclic GMP (cGMP). However, not all actions of NO appear to be mediated by cGMP (6,7,8).

Interleukin-1 (IL-1), a monocyte- and macrophage-derived cytokine, is believed to be a major inflammatory mediator in blood vessels. IL-1 stimulates the production of prostaglandin E_2 (PGE_2) by vascular smooth muscle cells (VSMC). Recent evidence has demonstrated that IL-1 also activates guanylate cyclase in VSMC by inducing production of NO (9,10). However, the role of endogenous NO induced by IL-1 in VSMC has not been fully elucidated. Recently, it has been reported that NO may act on cyclooxygenase, another enzyme containing the heme component, to mediate the norepinephrine-induced PGE_2 release from the hypothalamus (11). Therefore, to define the role of endogenous NO in VSMC, we examined whether NO induced by IL-1 participates in the activation of cyclooxygenase to stimulate PGE_2

[1]To whom correspondence should be addressed.

0006-291X/93 $4.00

production in VSMC. We found that NO may partially mediate the production of PGE_2 induced by IL-1 in VSMC.

MATERIALS AND METHODS

Materials: Human recombinant interleukin-1β (provided by Otsuka Pharmaceutical Co., Tokushima, Japan) was used in this study. The specific activity (LAF activity) of the IL-1β was $2x10^7$ half-maximal units/mg protein. N^G-monomethyl-L-arginine (L-NMMA), L-arginine, superoxide dismutase (SOD), sodium nitroprusside (SNP), 8-bromo-cyclic GMP (8Br-cGMP), and indomethacin were obtained from Sigma Chemical Co.

Cell culture: VSMC were isolated from rat aorta by explant method. Aortae were removed aseptically and placed in phosphate buffered saline, and the endothelium and adventitia were removed. Each aorta was cut into small pieces and cultured in DMEM with 20% FCS. Out grown cells were then passaged by harvesting with trypsin-EDTA. The cells were positively identified as smooth muscle cells by indirect immunofluorescent staining for α-actin antibody and anti-mouse IgG FITC conjugate (Sigma Chemical Co.). For experiments, cells were grown in DMEM supplemented with 10% FCS and antibiotics, and were used between passage 3 and 8.

Nitrite and prostaglandin E_2 assay: Confluent cultures of VSMC in 24-well plates were preincubated with serum-free DMEM containing 0.1% BSA at pH 7.4 for 48 h. Then the medium was replaced with new DMEM containing 0.1% BSA and the appropriate stimuli or modulating compounds, and incubated for the indicated time periods. The conditioned incubation medium was collected and centrifuged at 500 x g. The nitrite level in the cell-free supernatant was determined by the addition of an equal volume of Griess reagent (1% sulfanilamide and 0.1% naphthylethylenediamine dihydrochloride in 2% phosphoric acid). The absorbance at 540 nm was measured, and nitrite concentration was determined using sodium nitrite as a standard. Prostaglandin E_2 concentration in the cell-free supernatant was determined with an EIA kit (Amersham).

Data analysis: Significance of differences was determined by Student's paired t-test, and $p < 0.05$ was considered statistically significant.

RESULTS

Figure 1 shows the kinetics of NO (Fig. 1A) and PGE_2 (Fig. 1B) release from VSMC following exposure to 10 ng/ml IL-1 in the presence or absence of 3mM L-NMMA, an inhibitor of NO synthesis, at a higher concentration than that of L-arginine in the medium (0.4mM). NO release from VSMC was determined by measuring nitrite levels in the culture supernatant. Significant increases in NO levels were observed after 24 h and 48 h incubation with IL-1 (Fig. 1A). Coincubation with L-NMMA completely inhibited IL-1-induced NO release from VSMC. On the other hand, incubation of VSMC with IL-1 also induced a delayed and prolonged PGE_2 release. The kinetics of the PGE_2 release correlated with the kinetics of the NO production in IL-1-treated VSMC (Fig. 1B). Although L-NMMA alone did not affect the basal levels of PGE_2 release (data not shown), it partially but significantly inhibited IL-1-induced PGE_2 release from VSMC.

Based on the kinetics experiments, the 48 h time point was used in the following experiments designed to determine the relationship between NO and PGE_2 release from VSMC. As shown in Table 1, the inhibition of IL-1-induced NO and PGE_2 release by

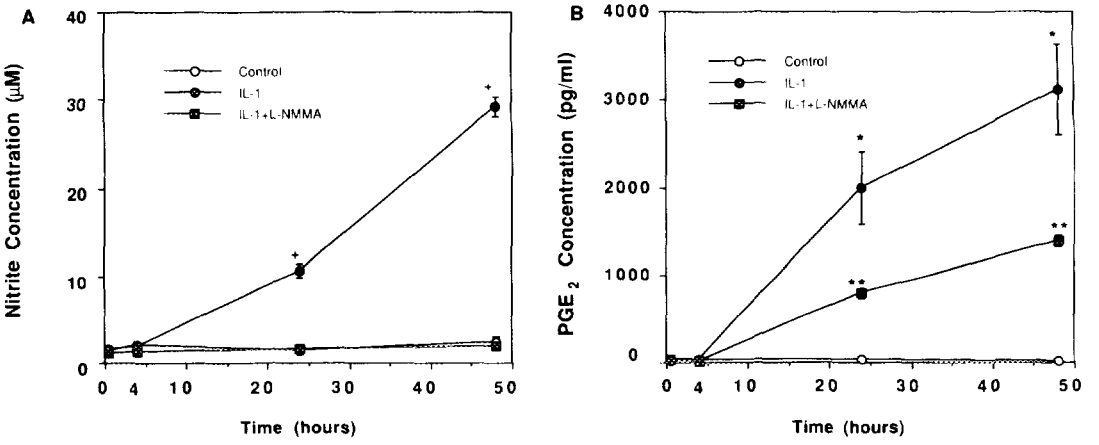

Figure 1. Kinetics of nitrite (A) and PGE_2 (B) release from VSMC. Confluent VSMC were preincubated for 48 h with DMEM containing 0.1% BSA, then cells were incubated with new medium containing IL-1 (10 ng/ml) in the presence or absence of L-NMMA (3mM). Incubation medium was then collected at the times indicated. Nitrite and PGE_2 concentrations in the medium were determined as described in the text. Values are mean±SD (n=3). $^{+}p < 0.005$, significantly different from control (A). $^{*}p < 0.05$, significantly different from control. $^{**}p < 0.05$, significantly different from VSMC treated with IL-1 alone (B).

L-NMMA was partially reversed by addition of 30mM L-arginine, a substrate for NO synthase. Although coincubation of VSMC with 1μM indomethacin, an inhibitor of cyclooxygenase, completely inhibited IL-1-induced PGE_2 release, it did not affect IL-1-

Table 1. Nitrite and PGE_2 release from VSMC

Treatment	Nitrite (μM)	PGE_2 (pg/ml)
Control	1.04 + 0.05	22.2 + 11.0
IL-1 (10 ng/ml)	20.52 ± 1.18^{+}	5693 ± 335*
IL-1 + L-NMMA (3mM)	1.37 + 0.16^{++}	2202 + 464**
IL-1 + L-NMMA + L-Arg (30mM)	6.02 + 0.37^{+++}	3610 + 533***
IL-1 + Indomethacin (1μM)	19.11 ± 0.69	29.6 ± 8.6
IL-1 + SOD (100 u/ml)	21.22 + 1.13	8487 + 379**
SNP (0.2mM)	14.80 + 0.16^{+}	288.5 + 6.5*
8Br-cGMP (2mM)	1.86 ± 1.14	24.4 ± 2.1

Confluent VSMC were preincubated with DMEM containing 0.1% BSA for 48 h, then the medium was replaced with new medium containing various drugs and incubated for 48 h. Nitrite and PGE_2 concentrations in the incubation medium were determined as described in the text. Values are mean±SD (n=3). $^{+}p < 0.001$, significantly different from control. $^{++}p < 0.001$, significantly different from VSMC treated with IL-1 alone. $^{+++}p < 0.001$, significantly different from VSMC treated with IL-1 and L-NMMA. $^{*}p < 0.001$, significantly different from control. $^{**}p < 0.001$, significantly different from VSMC treated with IL-1 alone. $^{***}p < 0.05$, significantly different from VSMC treated with IL-1 and L-NMMA.

induced NO production. In contrast, 100 u/ml SOD, which prolongs the half life of NO, significantly enhanced the IL-1-induced PGE_2 release. Furthermore, 0.2mM SNP, which releases NO directly, stimulated PGE_2 release from VSMC, although the amount of PGE_2 release by SNP was smaller than that by IL-1. On the other hand, 2mM 8Br-cGMP did not stimulate PGE_2 release from VSMC.

DISCUSSION

In this study we demonstrated that L-NMMA, an inhibitor of NO synthesis, partially inhibited PGE_2 release induced by IL-1 from VSMC. This inhibition was partially reversed by a 10-fold excess of L-arginine, a substrate for NO synthesis. Furthermore, SOD, which prolongs the half life of NO by inhibiting the generation of superoxide radicals, enhanced the IL-1-induced PGE_2 production. These findings suggest that NO may partially mediate the PGE_2 production induced by IL-1 in VSMC.

Although NO activates soluble guanylate cyclase by binding to its heme-component and stimulates cGMP accumulation in VSMC, 8Br-cGMP did not stimulate PGE_2 production. Furthermore, incubation with SNP alone resulted in an increase in PGE_2 production, suggesting that NO may directly stimulate the prostaglandin synthesis by binding to the heme-component of cyclooxygenase and activating this enzyme in VSMC. However, coincubation with L-NMMA did not completely inhibit the production of PGE_2 induced by IL-1, whereas it completely inhibited the IL-1-induced NO production. These results suggest that IL-1-induced PGE_2 production may be mediated through both NO-dependent and NO-independent mechanisms.

In this regard, IL-1 stimulates the activity of not only cyclooxygenase but also phospholipase A_2 (PLA_2), which stimulates the generation of arachidonic acid, a substrate for PGE_2 production, in human fibroblasts (12), rat mesangial cells (13), and rat VSMC (14). Since PLA_2 does not contain the heme-component, it is unlikely that NO stimulates PLA_2 activity in IL-1-treated VSMC. Thus, NO-independent mechanisms may be involved in the activation of this enzyme. Furthermore, although SNP stimulated both NO and PGE_2 release, the amount of PGE_2 release was much smaller than that induced by IL-1. Thus, other second signals may be involved in the full activation of the NO-dependent mechanism for PGE_2 production in IL-1-treated VSMC. However, further investigations are necessary to clarify these points.

In conclusion, our results indicate that NO could be one of the important second signals for the stimulation of PGE_2 production induced by IL-1 in VSMC.

ACKNOWLEDGMENTS

We thank Taeko Kaimoto for technical assistance. This work was supported by a Grant from Uehara Memorial Foundation.

REFERENCES

1. Moncada, S., Palmer, R.M.J., and Higgs, E.A. (1990) Pharmacol. Rev. 43, 109-142.
2. Snyder, S.H. (1992) Science 257, 494-496.
3. Bredt, D.S., Hwang, P.H., Glatt, C., Lowenstein, C., Reed, R.R., and Snyder, S.H. (1991) Nature (London) 351, 714-718.
4. Lyons, C.R., Orloff, G.J., and Cunningham, J.M. (1992) J. Biol. Chem. 267, 6370-6374.
5. Lamas, S., Marsden, P.A., Li, G.K., Tempst, P., and Michel, T. (1992) Proc. Natl. Acad. Sci. USA 89, 6348-6352.
6. Garg, U.C., and Hassid, A. (1991) J. Biol. Chem. 266, 9-12.
7. Macintyre, I., Zaidi, M., Alam, A.S.M.T., Datta, H.K., Moonga, B.S., Lidbury, P.S., Hecker, M., and Vane, J.R. (1991) Proc. Natl. Acad. Sci. USA 88, 2936-2940.
8. Lustig, H.S., Von Brauchitsch, K.L., Chang, J., and Greenberg, D.A. (1992) Brain Res. 577, 343-346.
9. Beasley, D., Schwartz, J.H., and Brenner, B.M. (1991) J. Clin. Invest. 87, 602-608.
10. Bernhardt, J., Tschudi, M.R., Dohi, Y., Gut, I., Urwyler, B., Bühler, F.R., and Lüscher, T.F. (1991) Biochem. Biophys. Res. Commun. 180, 907-912.
11. Rettori, V., Gimeno, M., Lyson, K., and McCann, S.M. (1992) Proc. Natl. Acad. Sci. USA 89, 11543-11546.
12. Lin, L.L., Lin, A.Y., and DeWitt, D.L. (1992) J. Biol. Chem. 267, 23451-23454.
13. Schalkwijk, C.G., De Vet, E., Pfeilschifter, J., and Van den Bosch, H. (1992) Eur. J. Biochem. 210, 169-176.
14. Nakano, T., Ohara, O., Teraoka, H., and Arita, H. (1990) FEBS Lett. 261, 171-174.