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European Journal of Pharmacology 311 (1996) 79–86

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Regulation of growth of cultured smooth muscle cells from diabetic rats by interleukin-1β: role of nitric oxide

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Received 21 September 1995; revised 6 March 1996; accepted 21 May 1996

Abstract

We examined the influence of streptozotocin-induced diabetes on the growth of cultured rat aortic smooth muscle cells in the presence of interleukin-1β. Interleukin-1β induced a dose-dependent biphasic effect on proliferation of diabetic and control smooth muscle cells, consistent with the data on [^{3}H]thymidine incorporation and cell counts. However, the major effect of interleukin-1β was to stimulate growth of diabetic cells and inhibit growth of control cells. Furthermore, interleukin-1β induced a dose-dependent increase in nitric oxide (NO) release and in intracellular cyclic GMP accumulation: nitrite release was similar in both smooth muscle cell models but cyclic GMP accumulation was greater in diabetic cells than in controls. These results suggest that the inhibitory loop involving NO is not effective enough to completely counterbalance the stimulatory effects of interleukin-1β on diabetic cells. Therefore, experimental diabetes may modify the interleukin-1β-regulated smooth muscle cell growth.

Keywords: Diabetes; Smooth muscle cell, vascular; Interleukin-1β; Nitric oxide (NO); Growth

1. Introduction

Diabetes mellitus is a major risk factor for atherosclerosis (Kannel and Mc Gee, 1978; Ruderman and Handenschild, 1984; Steiner, 1988). Despite the fact that many advances have been made in the understanding of the mechanisms of atherosclerosis in diabetes, new ways of research have been subject to increasing and widespread interest over the last years. For instance, the lesions could result from an excessive inflammatory-fibroproliferative response to various forms of insult to the endothelium and smooth muscle of the artery wall (Ross, 1993). Vascular smooth muscle cell proliferation in arterial intima is a critical event in the development of the atheromatous plaque (Ross, 1986). Cytokines and growth factors induce and regulate numerous critical cell functions. For instance, the cytokine production during the immune response could modulate vascular smooth muscle cell growth (Hansson et al., 1989; Libby, 1990).

One of these molecules, interleukin-1, is potentially important in cell proliferation (Bonin et al., 1989; Dinarello, 1985). Endothelium and vascular smooth muscle cells, in conjunction with macrophages, participate actively in interleukin-1 production during atherosclerotic processes (Moyer et al., 1991). Interleukin-1 has been implicated in the stimulation of vascular smooth muscle cell mitogenesis in culture (Libby et al., 1988; Raines et al., 1989). On the other hand, it has been recently demonstrated that interleukin-1 activates guanylate cyclase in vascular smooth muscle cells by inducing the production of nitric oxide (NO) (Beasley et al., 1991; Schini et al., 1991). NO is a key cell-signaling molecule, formed by enzymatic oxidation of guanidino nitrogen of L-arginine (Moncada et al., 1991). At present two major forms of NO synthase have been identified (Marletta, 1993; Nathan, 1992): a constitutive NO synthase and an inducible NO synthase. Nunokawa et al. (Nunokawa et al., 1993) have recently cloned inducible NO synthase in rat vascular smooth muscle cells. Furthermore, Kanno et al. (Kanno et al., 1993) demonstrated that interleukin-1β induced gene expression of inducible NO synthase in cultured rat smooth muscle cells. After exposure to cytokines, the amounts of NO generated by inducible NO synthase are greater than those generated by constitutive NO synthase. Both exogenous (Garg and Hassid, 1989) and interleukin-1β-induced NO (Scott-Burden et al., 1992) are known to inhibit vascular smooth muscle cell proliferation in culture, the former via a cyclic GMP production. Therefore, interleukin-1 could poten-

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PII S0014-2999(96)00398-6

tially both promote and inhibit vascular smooth muscle cell proliferation.

Thus, we were interested in the effect of experimental diabetes on vascular smooth muscle cell growth response to interleukin-1β. Therefore in this study, we compared the growth regulation by interleukin-1β and the role of NO in this process, in cultured aortic smooth muscle cells from streptozotocin-diabetic and non-diabetic rats.

2. Materials and methods

2.1. Materials

Streptozotocin, N^G-monomethyl-L-arginine (L-NMMA), aminoguanidine, ferrous hemoglobin and 3′-isobutyl-1-methylxantine (IBMX) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Interleukin-1β was obtained from Boehringer Mannheim (Meylan, France). Fetal calf serum was from Gibco/BRL (Sarl, Cergy Pontoise, France). Eagle's minimum essential medium (MEM) and all other reagents for cell culture were from Eurobio (Les Ulis, France).

2.2. Induction of diabetes

Diabetes was induced by a single injection of streptozotocin (66 mg/kg i.p.) in male Wistar rats. Control and diabetic animals were fed ad libitum for 5 weeks. Plasma glucose levels were: 34.9–40.3 mM in streptozotocin-diabetic rats and 9.6–9.9 mM in controls.

2.3. Cell culture

Thoracic aortas were excised from rats under sterile conditions, following a cervical dislocation and subjected to enzymatic and mechanical dissociation for the isolation of vascular smooth muscle cells, using a standard method (Bodin et al., 1991). The isolated cells were seeded into four-well plates (Nunc, Roskilde, Denmark) at 2×10^4 cells/cm^2 and grown in whole medium composed of MEM/Ham's F10 (in equal parts) supplemented with 2 mM glutamine, 0.05 mM vitamin C, 0.05 mM L-prolin, 100 U/ml penicillin G and 10% (v/v) fetal calf serum. The culture plates were incubated in a humidified incubator at 37°C in 5% CO_2 and 95% room air. Cells were positively identified as vascular smooth muscle cells by indirect immunofluorescence staining with anti-α actin antibody (Immunotech, Marseille, France). Primary and sub-cultures (one to three passages) were used for all experiments.

2.4. Determination of DNA synthesis

When vascular smooth muscle cells were subconfluent, they were synchronized to quiescence by a 48-h incubation in fresh medium supplemented with 0.5% fetal calf serum. They were then incubated 48 h with or without interleukin-1β or NO synthase inhibitors or NO inhibitor in fresh medium containing 5% fetal calf serum. We used inhibitors of NO synthase, L-NMMA and aminoguanidine (Marletta, 1993; Nathan, 1992), and an inhibitor of the effects of NO, hemoglobin (Archer, 1993; Murad et al., 1978) to further investigate the role of NO in interleukin-1β-modulated mitogenesis.

Cells were labeled with 0.225 μCi/well [^{3}H]thymidine (25 Ci/mmol; Amersham, Les Ulis, France) during the second 24 h of the 48-h incubation. After labeling, vascular smooth muscle cells were washed three times with cold phosphate buffer sodium, and treated with 10% trichloroacetic acid. The residues in the wells were solubilized in 0.3 N NaOH, and the radioactivity of the sample was counted by liquid scintillation spectroscopy. Cell number was determinated from a parallel set of cultures. Results were expressed as [^{3}H]thymidine incorporation rate per 10^6 cells for comparative purposes.

2.5. Determination of cell proliferation

Effects of interleukin-1β on cell proliferation was assessed by cell counts. Cells were cultured for 2 days in whole culture medium and they were then incubated for 2 days in the medium with 0.5% fetal calf serum. This was followed by a 4-day culture in the culture medium with or without increasing interleukin-1β concentrations. Culture media were changed daily; on appropriate days, the cells were harvested with trypsin-EDTA and counted using a counter (Coulter Epics XL; Coultronics, Margency, France).

2.6. Determination of intracellular cyclic GMP

Subconfluent cells rendered quiescent as previously described were incubated in whole culture medium with or without drugs. A phosphodiesterase inhibitor, IBMX (1 mM), was added to each well 30 min before the end of 24-h incubation. After the incubation, the medium was removed by aspiration and the cellular cyclic GMP extracted with 0.1 N HCl was determined by radioimmunoassay (Amersham).

2.7. Nitrite assay

Since NO in aqueous solution containing oxygen is oxidized primarily to nitrite with little or no formation of nitrate (Ignarro et al., 1993), we measured the nitrite level in the 24-h incubation medium as a reflection of the NO production. The nitrite level in cell-free supernatant was determined by adding an equal volume of Griess reagent (1% sulfanilamide and 0.1% naphthylethylene-diamine-dihydrochloride in 2% phosphoric acid). The absorbance at 540 nm was measured, and the nitrite concentration was determined using sodium nitrite as a standard.

2.8. Determination of lactate dehydrogenase activity

The effect of interleukin-1β on cell viability was examined by measuring lactate dehydrogenase (LDH) release. Quiescent vascular smooth muscle cells were incubated for 48 h with interleukin-1β (1 and 10 ng/ml). After the incubation, the medium was collected and the LDH activity in aliquots of cell-free supernatant was measured spectrophotometrically at 340 nm using a commercial kit (Sigma). Total LDH activity was determinated from the supernatant of a sample treated with 1% Triton X-100 for 30 min. Results were expressed as percentages of total LDH activity.

2.9. Data analysis

Results were expressed as means ± S.E.M. Significance of differences was determined by Student's *t*-test, and $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effect of interleukin-1β on [^{3}H]thymidine incorporation

Interleukin-1β induced a dose-dependent (0.01–10 ng/ml) biphasic effect on [^{3}H]thymidine incorporation in vascular smooth muscle cells from non-diabetic and diabetic rats (Fig. 1). In non-diabetic vascular smooth muscle cells (Fig. 1a), interleukin-1β at low concentrations (0.03 and 0.1 ng/ml) resulted in a moderate but significant increase in serum-induced mitogenesis. At concentrations higher than 0.3 ng/ml we observed an increasing inhibitory effect on [^{3}H]thymidine incorporation, the maximal decrement from baseline reaching a value of 35%. In diabetic vascular smooth muscle cells (Fig. 1b), interleukin-1β stimulated [^{3}H]thymidine incorporation with a peak value at 0.1 ng/ml. At 0.3 and 1 ng/ml, interleukin-1β produced a weaker stimulatory effect on [^{3}H]thymidine incorporation than at 0.1 ng/ml. No changes in serum-induced mitogenesis were observed at 3 and 10 ng/ml, compared with baseline. However, two main differences were observed between diabetic rats and control animals. In vascular smooth muscle cells from diabetic rats, the maximal increment in [^{3}H]thymidine incorporation with interleukin-1β was approximately 3 times higher than in vascular smooth muscle cells from controls. Furthermore, at higher tested concentrations (3 and 10 ng/ml), interleukin-1β did not inhibit [^{3}H]thymidine incorporation in diabetic vascular smooth muscle cells.

Moreover, basal thymidine incorporation by vascular smooth muscle cells from diabetic rats was nearly two times higher than basal thymidine incorporation by vascular smooth muscle cells from non-diabetic rats.

In the presence of L-NMMA or aminoguanidine, the thymidine incorporation induced by interleukin-1β at doses ranging from 1 to 10 ng/ml was shifted upward in non-diabetic vascular smooth muscle cells: 1 ng/ml interleukin-1β resulted in stimulation of [^{3}H]thymidine incorporation and the inhibition of [^{3}H]thymidine incorporation induced by 10 ng/ml interleukin-1β was prevented. Furthermore, these inhibitors increased stimulation of [^{3}H]thymidine incorporation induced by 0.1 ng/ml interleukin-1β in non-diabetic cells (Fig. 2a). On the other hand, Fig. 2b indicated that in diabetic vascular smooth muscle cells, these inhibitors increased stimulation of [^{3}H]thymidine incorporation induced by 1 ng/ml interleukin-1β, and permitted an increase in [^{3}H]thymidine incorporation in response to 10 ng/ml interleukin-1β. However, maximal [^{3}H]thymidine incorporation induced by 0.1 ng/ml interleukin-1β was not modified by L-NMMA or aminoguanidine.

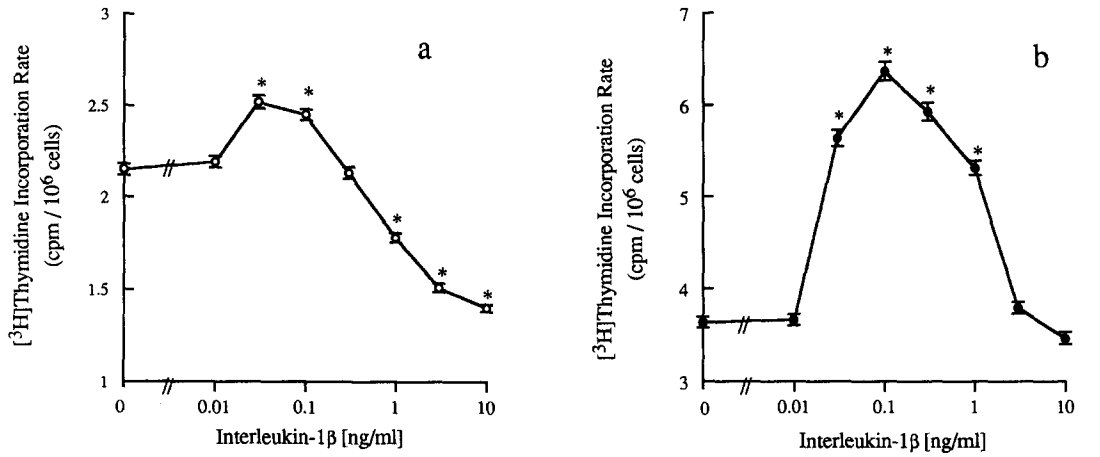

Fig. 1. Effect of interleukin-1β on [^{3}H]thymidine incorporation into DNA of vascular smooth muscle cells from non-diabetic (a) and streptozotocin-diabetic (b) rats. Results are expressed as means ± S.E.M. from four separate experiments, each performed in quadruplicate. * $P < 0.001$ versus values found in the absence of interleukin-1β.

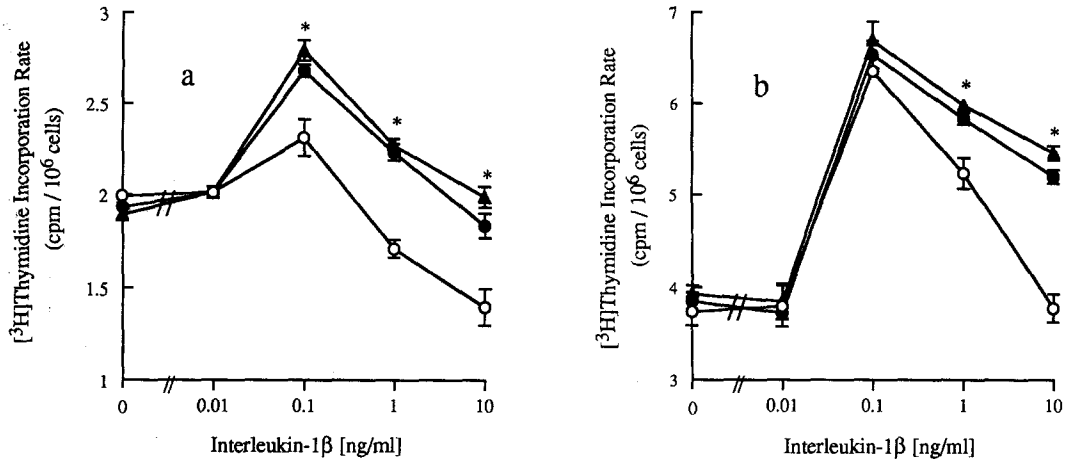

Fig. 2. DNA synthesis of vascular smooth muscle cells from non-diabetic (a) and streptozotocin-diabetic (b) rats in response to interleukin-1β alone (○) and with aminoguanidine (0.5 mM) (●) or L-NMMA (0.5 mM) (▲). Results are expressed as means ± S.E.M. from three separate experiments, each performed in quadruplicate. * $P < 0.001$ versus interleukin-1β.

As indicated in Table 1, coincubation with L-NMMA, aminoguanidine or hemoglobin prevented the inhibition of [^{3}H]thymidine incorporation induced by 3 ng/ml interleukin-1β in non-diabetic vascular smooth muscle cells. On the other hand, coincubation with these inhibitors permitted an increase in [^{3}H]thymidine incorporation by diabetic vascular smooth muscle cells (up to 46%) in response to interleukin-1β. L-NMMA, aminoguanidine and hemoglobin by themselves did not affect the basal [^{3}H]thymidine incorporation by vascular smooth muscle cells from both non-diabetic and diabetic rats.

3.2. *Effect of interleukin-1β on vascular smooth muscle cell proliferation*

As depicted in Fig. 3, interleukin-1β resulted in a dose-dependent (0.01–10 ng/ml) biphasic effect on proliferation of vascular smooth muscle cells from non-diabetic and diabetic rats. Interleukin-1β induced an increase in vascular smooth muscle cell number in both diabetic and non-diabetic rats. However, stimulating action of interleukin-1β was observed at 0.03–1 ng/ml in diabetic cells and only at 0.03–0.1 ng/ml in control cells. Moreover, diabetic smooth muscle cell number was increased more (up to 33%) than non-diabetic smooth muscle cell number (up to 12%). Inhibitory action of interleukin-1β on non-diabetic smooth muscle cell proliferation was not observed on diabetic smooth muscle cell proliferation.

Furthermore, the effect of interleukin-1β on LDH release was examined. The results showed no significant increase in LDH activity in culture medium from non-diabetic vascular smooth muscle cells: 14 ± 0.2% (without interleukin-1β), 22 ± 0.65% (with 1 ng/ml interleukin-1β), 26 ± 0.4% (with 10 ng/ml interleukin-1β). In the same way, LDH activity in culture medium of diabetic vascular smooth muscle cells was: 12 ± 0.5%, 13 ± 0.25%, 14 ± 0.3%, without interleukin-1β, with 1 ng/ml interleukin-1β and with 10 ng/ml interleukin-1β, respectively.

Table 1
Effect of NO synthesis inhibitors and hemoglobin on [^{3}H]thymidine incorporation by streptozotocin-diabetic and non-diabetic vascular smooth muscle cells in the presence of 3 ng/ml interleukin-1β

Effector	Thymidine incorporation (% of control)	
	Streptozotocin-diabetic vascular smooth muscle cells	Non-diabetic vascular smooth muscle cells
L-NMMA	104.53 ± 1.64	93.30 ± 1.6
Aminoguanidine	107.96 ± 4.44	98.15 ± 0.9
Hemoglobin	100.85 ± 1.88	102.5 ± 1.5
Interleukin-1β	104.53 ± 1.64	66.47 ± 3.26
Interleukin-1β + L-NMMA	146.13 ± 3.73 [a]	99.98 ± 2.60 [a]
Interleukin-1β + aminoguanidine	138.73 ± 3.74 [a]	90.67 ± 1.54 [a]
Interleukin-1β + hemoglobin	141.06 ± 3.26 [a]	98.45 ± 3.13 [a]

Control was defined as [^{3}H]thymidine incorporation induced by 5% serum. Concentrations of effectors were as follows: 0.5 mM L-NMMA, 0.5 mM aminoguanidine, 10 μM hemoglobin. Results are expressed as means ± S.E.M. from three separate experiments, each in quadruplicate. [a] $P < 0.001$ versus interleukin-1β.

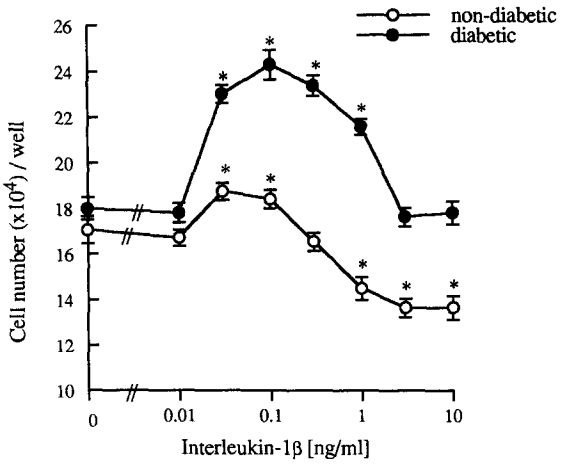

Fig. 3. Effect of interleukin-1β on proliferation of vascular smooth muscle cells from non-diabetic and streptozotocin-diabetic rats. Results are expressed as means ± S.E.M. from three separate experiments, each performed in quadruplicate. * $P < 0.01$ versus values found in the absence of interleukin-1β.

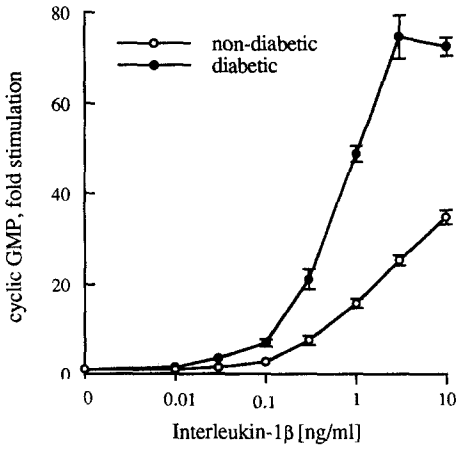

Fig. 5. Effect of interleukin-1β on cyclic GMP accumulation in vascular smooth muscle cells from streptozotocin-diabetic and non-diabetic rats. Results are expressed as fold stimulation over control incubation lacking interleukin-1β and represent means ± S.E.M. from three separate experiments, each performed in quadruplicate.

3.3. Effect of interleukin-1β on nitrite release

Interleukin-1β induced a dose-dependent nitrite release from both vascular smooth muscle cell models (Fig. 4). Nitrite levels were not different between vascular smooth muscle cells from non-diabetic and diabetic rats. Furthermore, L-NMMA and aminoguanidine produced each a marked ($P < 0.001$) attenuation of the interleukin-1β-induced nitrite release from diabetic and non-diabetic vascular smooth muscle cells. L-NMMA and aminoguanidine alone did not affect the basal levels of nitrite release.

3.4. Effect of interleukin-1β on cyclic GMP accumulation

Interleukin-1β increased the intracellular cyclic GMP content in a dose-dependent manner (Fig. 5). However, vascular smooth muscle cells from diabetic rats had a greater tendency to respond to interleukin-1β, at doses from 0.03 to 10 ng/ml, than those from non-diabetic rats. The cyclic GMP content of cells treated with 10 ng/ml interleukin-1β exhibited a two-fold increase in diabetic vascular smooth muscle cells compared with vascular smooth muscle cells from non-diabetic animals.

As shown in Fig. 6, the increase in cyclic GMP content in diabetic and non-diabetic vascular smooth muscle cells induced by 3 ng/ml interleukin-1β was significantly ($P < 0.001$) inhibited by coincubation with L-arginine analogue, L-NMMA.

4. Discussion

In this study we have shown, first that interleukin-1β induces a dose-dependent biphasic effect on DNA synthesis and proliferation of cultured vascular smooth muscle cells from streptozotocin-diabetic rats and, second, that the

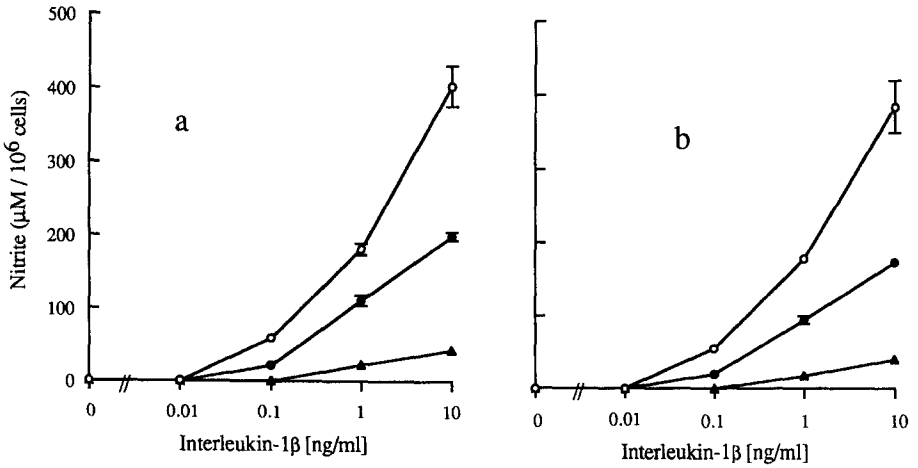

Fig. 4. Concentration-dependent production of nitrite in response to interleukin-1β alone (○) and with aminoguanidine (0.5 mM) (●) or L-NMMA (0.5 mM) (▲) by vascular smooth muscle cells from non-diabetic (a) and streptozotocin-diabetic (b) rats. Results are expressed as means ± S.E.M. from four separate experiments, each performed in quadruplicate.

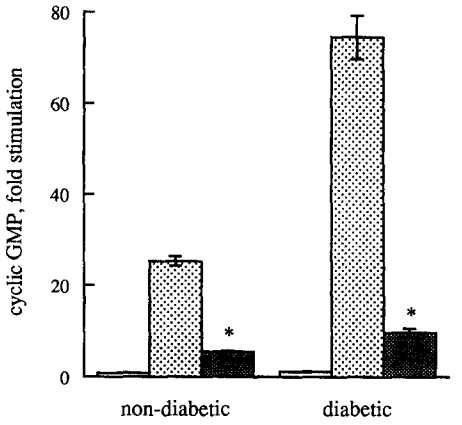

Fig. 6. Effect of L-NMMA (0.5 mM) on 3 ng/ml interleukin-1β-induced cyclic GMP accumulation in vascular smooth muscle cells from non-diabetic and streptozotocin-diabetic rats. Results are expressed as fold stimulation over control incubation lacking interleukin-1β and L-NMMA, and represent means ± S.E.M. from three separate experiments, each performed in quadruplicate. * $P < 0.001$ versus interleukin-1β. Blank column: L-NMMA; stippled column: interleukin-1β; cross-hatched column: interleukin-1β + L-NMMA.

NO released after interleukin-1β stimulation could modulate this growth.

In the first phase, interleukin-1β promoted growth whereas in the second phase this increase was progressively abolished. By contrast, in non-diabetic vascular smooth muscle cells, the first phase was markedly attenuated and the second phase showed an inhibition of growth. NO, a well-known agent for inhibiting cell proliferation (Garg and Hassid, 1989), seems to be markedly involved in vascular smooth muscle cell growth response to interleukin-1β. Indeed, interleukin-1β induced a dose-dependent increase in NO release from both vascular smooth muscle cell models. We showed that the elevation of nitrite levels in culture medium from non-diabetic vascular smooth muscle cells exposed to interleukin-1β was correlated with the inhibition of the growth. These data are in agreement with the studies of Scott-Burden et al. (Scott-Burden et al., 1992) performed in cultured rat and human vascular smooth muscle cells. It is well established that interleukin-1β is a potent inducer of NO synthesis in vascular smooth muscle cells (Beasley et al., 1991; Busse and Mülsch, 1990; Schini et al., 1991), although interleukin-1β is also known to have a mitogenic activity (Libby et al., 1985; Raines et al., 1989). Thus, our results suggest that interleukin-1β induces both stimulatory and inhibitory signals for DNA synthesis in streptozotocin-diabetic vascular smooth muscle cells (Rangnekar et al., 1991).

Therefore, we tested whether blockade of NO synthesis during incubation of vascular smooth muscle cells with interleukin-1β would unmask a mitogenic effect. In the presence of NO synthase inhibitors, L-NMMA (analogue of L-arginine) (Knowles and Moncada, 1994) and aminoguanidine (the more recently described inducible NO synthase selective inhibitor) (Corbett et al., 1992), interleukin-1β, even at high concentrations, stimulated diabetic vascular smooth muscle cell mitogenesis. Similar results were obtained with hemoglobin, an inhibitor of NO. Under the same conditions, interleukin-1β also stimulated non-diabetic vascular smooth muscle cell mitogenesis and the inhibition of mitogenesis induced by high concentrations of interleukin-1β was abolished. Moreover, the increased stimulation of non-diabetic smooth muscle cell mitogenesis induced by interleukin-1β (0.1 ng/ml) in the presence of L-NMMA suggests that the inhibitory loop, that involves NO, was already effective to reduce the stimulatory effects of interleukin-1β. By contrast, NO did not seem effective enough to reduce the mitogenic effect of interleukin-1β at 0.1 ng/ml on diabetic smooth muscle cells, because in the presence of L-NMMA the mitogenic effect of interleukin-1β was not increased.

Furthermore, interleukin-1β induced concomitant increases in intracellular cyclic GMP in vascular smooth muscle cells from streptozotocin-diabetic and non-diabetic rats. The presence of IBMX suggests that interleukin-1β activates guanylate cyclase rather than inhibiting phosphodiesterase. L-NMMA attenuated interleukin-1β-induced increases in cyclic GMP in both vascular smooth muscle cell models. This suggests that interleukin-1β activates soluble guanylate cyclase through the stimulation of NO production (Beasley et al., 1991). However, the cyclic GMP production by streptozotocin-diabetic vascular smooth muscle cells was significantly enhanced when compared to the non-diabetic vascular smooth muscle cells. Thus, our results seem to suggest that, in streptozotocin-diabetic rats, the inhibitory loop that involves NO is not effective enough, first to completely counterbalance the stimulatory effects of interleukin-1β and, second, to mediate the interleukin-1β-induced inhibition of mitogenesis.

It has been previously reported (Raines et al., 1989) that mitogenic activity of interleukin-1 for vascular smooth muscle cells in culture may be mediated by induction of platelet-derived growth factor (PDGF)-AA, in cooperation with such other growth factors as basic fibroblast growth factor (Gay and Winkles, 1991; Majack et al., 1990), another strong mitogen for vascular smooth muscle cells (Klagsbrun and Edelman, 1989). These growth factors may contribute to the mitogenic cascade elicited by the cytokine. Moreover, Kawano et al. (Kawano et al., 1993) have shown that cultured vascular smooth muscle cells from streptozotocin-diabetic rats and alloxan-diabetic rabbits over-react on PDGF stimulation through over-expression of the PDGF β-receptor gene. Therefore, an over-response to growth factors could partly explain the interleukin-1β promoted DNA synthesis as observed in streptozotocin-diabetic vascular smooth muscle cells. Moreover, our results also showed that basal [^{3}H]thymidine incorporation by vascular smooth muscle cells was enhanced in streptozotocin-diabetic rats when compared to non-diabetic rats. Alipui et al. (Alipui et al., 1993) also showed an increased proliferation of cultured smooth muscle cells

from alloxan-diabetic rabbits. An enhanced responsiveness of these cells to growth factors present in culture medium could explain these results. Moreover, we have found that diabetic smooth muscle cells in culture expressed a more dedifferentiated state (unpublished data) which could also explain enhanced responsiveness to growth factors like PDGF.

The regulation of cultured vascular smooth muscle cell growth by interleukin-1β could also include inhibitory loops that involve other second messengers besides cGMP. Interleukin-1β is known to stimulate the production of growth inhibitory prostanoids (Libby et al., 1988). More recently, it has been shown that NO could directly activate cyclooxygenase enzymes (Salvemini et al., 1993) and could partially mediate interleukin-1-induced prostaglandin E_2 production in cultured vascular smooth muscle cells (Inoue et al., 1993). Since coincubation with indomethacin (3 μM), a cyclooxygenase inhibitor, does not affect the growth response of non-diabetic and streptozotocin-diabetic vascular smooth muscle cells to interleukin-1β (data not shown), it is suggested that this inhibitory pathway is only poorly involved in interleukin-1β growth regulation.

Impairment in either NO synthesis or cyclic GMP effect could explain why interleukin-1β does not inhibit diabetic smooth muscle cells. Ikeda et al. (Ikeda et al., 1995) suggested that an increase in intracellular calcium ion enhances interleukin-1β-induced NO synthesis in vascular smooth muscle cells. However, we found that NO production induced by interleukin-1β was not different in diabetic and non-diabetic cells. Therefore, an impairment in calcium dependent or independent NO synthesis induced by interleukin-1β does not explain the response of diabetic smooth muscle cells to interleukin-1β. Other studies suggest that the cyclic GMP-dependent activation of cyclic AMP kinase may be in part responsible for the NO-dependent inhibition of proliferation of rat aortic smooth muscle cells (Cornwell et al., 1994). We have shown in a previous study (Etienne et al., 1996) that 8-bromo-cyclic GMP, a lipid-soluble analogue of cyclic GMP, exhibited the same dose-dependent antimitogenic effect in non-diabetic and diabetic cultured smooth muscle cells. Therefore, an altered responsiveness to cyclic GMP or a modified activation of kinase by cyclic GMP did not seem to be involved in diabetic smooth muscle cells. On the other hand, we have previously tested the effect of such NO-generating agents as sodium nitroprusside and *S*-nitroso-*N*-acetylpenicillamine, on diabetic cultured smooth muscle cells (Etienne et al., 1996). Our results showed that diabetic smooth muscle cell responsiveness to NO was enhanced through an increased cyclic GMP production. We found in the present study that cyclic GMP production stimulated by NO-induced interleukin-1β was also increased in diabetic smooth muscle cells. Therefore, these results suggest that an over-sensibility of diabetic smooth muscle cells to growth factors could explain the stimulatory effects of interleukin-1β.

It is important to point out that the reduction of [^{3}H]thymidine incorporation, as demonstrated in the present work, is not related to a cytotoxic action of NO, since incubation of the cells with higher concentrations of interleukin-1β, for 48 h, did not result in a significant stimulation on the LDH release into the medium. Some studies (Ellman et al., 1993; Fukuo et al., 1995; Pinsky et al., 1995) have shown that NO exerts a cellular cytotoxicity, that probably resulted from prolonged incubation with interleukin-1β.

In conclusion, NO seems to play an important role in interleukin-1β-regulated vascular smooth muscle cell growth. Its effect would have the obvious advantage of limiting uncontrolled cell proliferation in response to mitogenes. Our results show that experimental diabetes might induce an impairment in interleukin-1β growth regulation. In streptozotocin-diabetic rats, interleukin-1β, which is a major cytokine involved in the atherosclerosis process, stimulates the proliferation of vascular smooth muscle cells and thereby may partially contribute to the development or the worsening of atherosclerotic lesions in diabetes.

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