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Differential regulation of inducible nitric oxide synthase by fibroblast growth factors and transforming growth factor β in bovine retinal pigmented epithelial cells: Inverse correlation with cellular proliferation

(retina)

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ABSTRACT **Bovine retinal pigmented epithelial (RPE) cells express, after activation with interferon γ (IFN-γ) and lipopolysaccharide (LPS), an inducible nitric oxide synthase (NOS). Experiments were done to investigate the effects of the transforming growth factor β1, epidermal growth factor, and fibroblast growth factors (FGFs), which are abundant in the retina, on NOS activity. Transforming growth factor β1 slightly increases the production of nitrite, an oxidation product of NO, induced by LPS plus IFN-γ, whereas acidic and basic FGFs markedly inhibit the nitrite release due to LPS/IFN-γ in a concentration-dependent manner, and epidermal growth factor did not modify LPS/IFN-γ-induced NOS activity. The growth factors alone did not stimulate nitrite release. We have attempted to elucidate the mechanism of FGF inhibition. Results with heparin, suramin, and tyrphostin suggest involvement of the high-affinity receptor for FGF in its inhibition of LPS/IFN-γ-stimulated NOS activity. Continued stimulation of RPE cells with LPS/IFN-γ was essential for the induction of NO synthesis, and maximal inhibition was obtained when FGF was present during stimulation with LPS/IFN-γ, suggesting that FGF inhibits NOS induction. Furthermore, an antiproliferative action of NO was demonstrated by an inverse correlation between the amounts of nitrite or citrulline produced in response to different stimuli (LPS/IFN-γ or LPS/IFN-γ with growth factors) and the level of cellular proliferation. Similar inhibition of growth was obtained when RPE cells were incubated with an NO donor, sydnonimide. Because NO acts as a cytotoxic compound in the retina, FGF, by inhibiting the induction of NOS in RPE cells, may have beneficial effects in protecting the retina from cytokine and endotoxin-mediated tissue damage.**

Arginine-dependent NO formation is an important autocrine and paracrine signaling pathway that plays important roles in the physiology of numerous mammalian cells (for review, see ref. 1). Characterization of the enzymes responsible for NO generation has classified them into two groups: constitutive and inducible NO synthases (NOSs) (2). The constitutive isoforms, which have been recently cloned (3, 4), are primarily expressed in endothelial and some neural cells, and their activity is dependent on calcium and calmodulin (1, 2). Concerning the inducible form, its activity does not require calcium. Inducible isoforms have been described in many cell types, including hepatocytes (5), smooth muscle (6), mesangial (7), and endothelial cells (8), but only the macrophage isoform has been cloned (9, 10). In macrophages, the production of NO begins several hours after stimulation by endotoxin and cytokines, and the sustained synthesis of NO accounts for their cytotoxic properties (antitumoral and antimicrobial) (1, 11). The large production of NO can be also deleterious and may be important in some pathological states (1, 12, 13).

In the retina, there is evidence for the existence of NOS in different cell types. A constitutive activity, which exhibited the same characteristic as the brain isoform, was detected in the neurosensorial retina (14–16). Furthermore, we recently showed that the bovine retinal pigmented epithelial (RPE) cells can express a macrophage type of NOS after endotoxin and cytokine treatment (17). The distinct functions of these NOS isoforms have not been defined. RPE cells constitute an epithelial monolayer located between the choroidal blood circulation and the neurosensory retina and are involved in the nurturing and renewal of the photoreceptor cell layer (18). RPE cells exhibit similar properties as macrophages, including phagocytic activity, expression of major histocompatibility complex II antigens after interferon γ (IFN-γ) stimulation (19) and also expression of an inducible NOS activity (17). In macrophages, NOS induction can be modulated by growth factor members of the transforming growth factor β (TGF-β) family (20). In recent years, RPE cells have been shown to secrete or be sensitive to numerous growth factors, including epidermal growth factor (EGF), TGF-β, and particularly fibroblast growth factors (FGFs) (21–23), which are important molecules in the development, normal functioning, and survival of photoreceptors (21, 24). In the present study, we investigated the effects of different growth factors, particularly FGFs, on NO production, and we have examined the consequences of a sustained NO release on the proliferation of RPE cells.

MATERIALS AND METHODS

Cell Cultures. The bovine RPE cells were prepared as reported (17, 24). Cells were cultivated in Dulbecco's modified essential medium (DMEM)/10% fetal calf serum (GIBCO/BRL)/fungizone at 2.5 μg/ml/gentamycin at 50 μg/ml/2 mM L-glutamine. Cells of passages 1–5 were used for experiments.

Chemicals and Cytokines. L-Arginine was obtained from Merck. N^G-Monomethyl-L-arginine and tyrphostin were from Calbiochem. Lipopolysaccharide (LPS) from *Salmo-*

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Abbreviations: NOS, nitric oxide synthase; RPE, retinal pigmented epithelial; IFN-γ, interferon γ; LPS, lipopolysaccharide; aFGF, acidic fibroblast growth factor; bFGF, basic fibroblast growth factor; EGF, epidermal growth factor; TNF-α, tumor necrosis factor α; TGF-β, transforming growth factor β; SIN-1, 3-morpholinosydnonimine; SIN-1C, *N*-morpholinoimminoacetonitryle.

nella typhymurium, sulfanilamide, naphthylethylenediamine, and NADPH were from Sigma. (6*R*)-5,6,7,8-Tetrahydro-L-biopterin dihydrochloride (BH_4) was obtained from B. Schircks Laboratories (Jona, Switzerland). Suramin was obtained from Bayer France, and heparin was from Sanofi (Paris). L-[2,3,4,5-^{3}H]Arginine monohydrochloride was obtained from Amersham. Sydnonimine analogues (SIN-1 and SIN-1C) were from Hoechst Laboratories through S. Harbon (Centre National de la Recherche Scientifique URA 1131, Orsay, France). TGF-β1 was purchased from Realef (Paris), and EGF was from Collaborative Research. Human recombinant basic FGF (bFGF) was prepared by Carlo Erba (Italy). Human recombinant acidic FGF (aFGF) was prepared in the laboratory with the collaboration of D. Mayaux (Rhône-Poulenc Rorer, Paris). Human recombinant tumor necrosis factor α (TNF-α) was from A. Bousseau (Rhône-Poulenc Rorer). Bovine recombinant IFN-γ was provided by T. Ramp (CIBA–Geigy).

Formation of Nitrite. At subconfluency, RPE cells in 6-well culture plates were treated with LPS and IFN-γ, with or without different growth factors, in fresh DMEM/10% fetal calf serum and supplemented with 2 mM L-arginine. After incubation for the time indicated in each specific experiment, nitrite concentration was determined in cell-free culture supernatants, as described (17, 25).

Cell Proliferation Assay. The RPE cells were plated into 6-well culture plates and were grown in DMEM/10% fetal calf serum. The following day, cells were treated with the different agents (LPS, IFN-γ, growth factors, or sydnonimine analogues) in fresh DMEM/10% fetal calf serum. After incubation for the time indicated in each specific experiment, cells were washed, and the remaining adherent cells were trypsinized and counted. Duplicate dishes were used for each experimental point.

Assay of NOS in RPE Cell Extracts. Subconfluent cells were incubated for 72 hr with or without stimulatory agents (LPS, IFN-γ, bFGF, or TGF-β). Cells were harvested by trypsinization, washed once in phosphate-buffered saline (PBS) and resuspended in 50 mM Tris·HCl/10 mM dithiothreitol (pH 7.8) buffer. After sonication, the homogenate was centrifuged for 20 min at 4°C at 100,000 × *g*. The supernatant was used to evaluate NOS activity by the conversion of L-[^{3}H]arginine to L-[^{3}H]citrulline (25) after determination of protein content (Bio-Rad assay kit). Samples of different cell extracts were incubated for 15 min at 37°C in a solution of 50 mM Tris·HCl, pH 7.8, containing 10 mM dithiothreitol, 0.15 mM NADPH, 0.1 mM (6*R*)-5,6,7,8-tetrahydro-L-biopterin dihydrochloride, and 5 μM L-arginine with L-[^{3}H]arginine. In some experiments, 0.1 mM N^G-monomethyl-L-arginine was added simultaneously. The reaction was stopped by heating to 90°C for 2 min, and 20 mM sodium citrate, pH 2.2, was added to the precipitates. After centrifugation, a 100-μl aliquot of the supernatant was loaded onto a strong cation-exchange column (Zorbax 300 SCX, Societé Française de chromatographie sur colonne/Shandon) as in ref. 25.

Statistical Analysis. Results are expressed as mean ± SEM and were analyzed statistically by using Student's *t* test. *P* values <0.05 were considered as significant.

RESULTS

Different Effects of aFGF, bFGF, EGF, and TGF-β on NO Production Induced by IFN-γ Plus LPS. RPE cells activated with IFN-γ (100 units/ml) and LPS (1 μg/ml) produced high levels of NO, measured as nitrite in the culture supernatants, as described (17). When cells were coincubated with aFGF or bFGF and NOS inducers (LPS/IFN-γ) for 72 hr, the production of nitrites was markedly reduced. The inhibitions were dose-dependent. bFGF was the most potent inhibitory agent, with half-maximal inhibition (EC_{50}) at 0.2 ng/ml, compared with aFGF, which had an EC_{50} at 1 ng/ml (Fig. 1). Maximal inhibition (percentage inhibition of nitrite release compared with control) was achieved with bFGF at 10 ng/ml (93 ± 6.4%) or aFGF at 100 ng/ml (91 ± 3.8%). As with LPS and IFN-γ, NO production induced by IFN-γ and TNF-α (17) was sensitive to suppression by bFGF with 87 ± 7% inhibition of nitrite release compared with control (data not shown). The presence of TGF-β in addition to IFN-γ and LPS in culture medium significantly increased NO synthesis, as compared with LPS/IFN-γ alone, for concentrations >1 ng/ml (Fig. 2). Maximal stimulation was obtained with TGF-β at 10 ng/ml, corresponding to 47 ± 3.9% increase compared with control (IFN-γ and LPS in the absence of TGF-β). Nitrite production induced by the combination of LPS plus TNF-α (17) was also sensitive to TGF-β (data not shown) with a 23 ± 2.4% increase of nitrite release compared with control ($P < 0.05$). TGF-β added alone to RPE cells did not induce nitrite release (see below, Fig. 4). The third growth factor used, EGF, a stimulator of RPE cell proliferation like

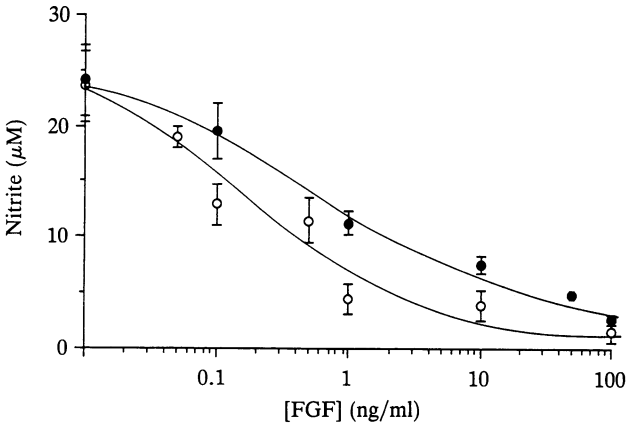

FIG. 1. Dose-dependent inhibition of nitrite production due to LPS/IFN-γ by bFGF and aFGF in RPE cells. RPE cells were incubated with IFN-γ at 100 units/ml plus LPS at 1 μg/ml and with the indicated concentrations of aFGF (●) or bFGF (○). After 72 hr, culture media were assayed for nitrite. Values are means ± SEMs for four different experiments, each done in duplicate.

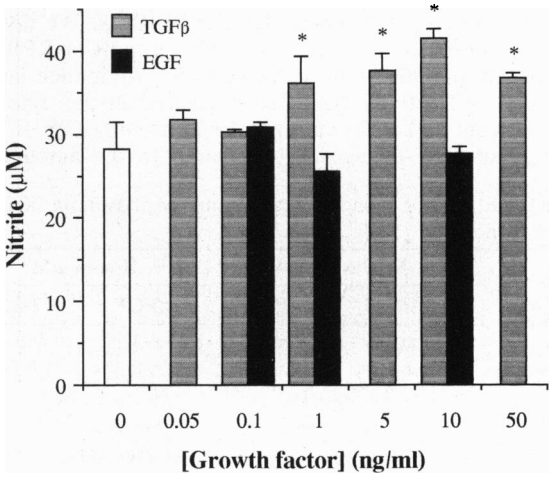

FIG. 2. Effect of increased concentrations of TGF-β and EGF on nitrite release from RPE cells activated by LPS plus IFN-γ. RPE cells were costimulated with IFN-γ at 100 units/ml, LPS at 1 μg/ml, and the indicated concentrations of EGF or TGF-β. After 72 hr, culture media were assayed for nitrite. Values are means ± SEMs for three different experiments, each done in duplicate. Asterisks denote statistically significant differences from control (nitrite release due to LPS/IFN-γ in absence of growth factor).

FGFs (23), did not affect the production of NO induced by LPS/IFN-γ in RPE cells (Fig. 2).

Modulation of FGF and TGF-β Effects on LPS/IFN-γ-Stimulated Nitrite Production in RPE Cells. To investigate the action of FGFs and TGF-β on the regulation of NO synthesis induced by LPS/IFN-γ, we tested different agents known to modulate the biological effects of growth factors by means of direct interaction with the growth factor or with its receptors. In RPE cells, heparin, a modulator of growth factor activities (26, 27), did not affect the inhibitory response of bFGF on NO synthesis but increased aFGF-induced attenuation of nitrite release (Table 1). Suramin, a polyanionic compound that antagonizes some growth factor-mediated responses (26), and tyrphostin, a tyrosine kinase inhibitor (28), partially suppress bFGF inhibition, whereas they have no significant effect on TGF-β response (Table 1). On the other hand, it was interesting to note that tyrphostin slightly decreased the LPS/IFN-γ-induced NO production (Table 1). The possible involvement of tyrosine phosphorylation in the cellular response induced by LPS/IFN-γ may explain this observation (29).

Dependence of Inhibition of LPS/IFN-γ-Induced Nitrite Production on Period of FGF Exposure. Maximal inhibition of LPS/IFN-γ-induced nitrite production (96.5 ± 1.8%) was achieved when bFGF was present during the entire incubation with LPS/IFN-γ (Fig. 3*A*). Twenty-four-hour preincubation of RPE cells with bFGF before LPS/IFN-γ was less effective (only 52 ± 3.5% inhibition), and when bFGF was added 12, 24, or 36 hr after IFN-γ/LPS, an inhibition of nitrite production could also be detected but was always inferior to the control (LPS/IFN-γ and bFGF added together). The minimum time for detecting NO generation in LPS/IFN-γ-stimulated RPE cells is from 18 to 24 hr, after which synthesis of NO continues for at least 96 hr (17). To characterize the induction phase of NOS, RPE cells were incubated with LPS/IFN-γ, and after various times the cells were washed and reincubated in fresh DMEM without IFN-γ and LPS. Results shown in Fig. 3*B* demonstrate that the level of nitrites after 72 hr seems directly proportional to the time of exposure to IFN-γ and LPS. One explanation that could account for these results is that NO synthesis by LPS/IFN-γ requires continued stimulation of the RPE cells with endotoxin and cytokine, as suggested for mouse macrophages (30).

Correlation Between Nitrite Production, Citrulline Synthesis, and Decrease of Proliferation of RPE Cells. We investigated the effect of LPS/IFN-γ on the growth of RPE cell culture, in parallel with their capacity to induce nitrite release. NOS activity was also measured in the cytosolic extracts from RPE cells cultured for 72 hr with LPS/IFN-γ, bFGF, and TGF-β and was compared to the amounts of

Table 1. Effects of heparin, suramin, and tyrphostin on FGF and TGF-β responses

Addition	Nitrite release from LPS + IFN-γ, μM			
	Control	+ bFGF	+ aFGF	+ TGF-β
None	22.7 ± 1.6	3.7 ± 0.3	8.4 ± 2.1	30.4 ± 2.95
Heparin	21.5 ± 2.6	2.5 ± 0.7	1.8 ± 0.2*	ND
Suramin	23.1 ± 2.4	13.1 ± 1.9*	ND	31.8 ± 3.90
Tyrphostin	16.75 ± 3†	14.2 ± 2.4*	ND	27.4 ± 1.05

RPE cells were incubated with IFN-γ (100 units/ml) and LPS (1 μg/ml) with or without bFGF (10 ng/ml), aFGF (10 ng/ml), or TGF-β (10 ng/ml). Heparin (10 μg/ml), suramin (50 μg/ml), or tyrphostin (50 μM) were added simultaneously with LPS/IFN-γ. After 72 hr, nitrite content was evaluated in the medium. Values are means ± SEMs for three different experiments, each done in duplicate. ND, not done.

*Significantly different from the samples treated with growth factor ($P < 0.01$).

†Significantly different from control treated ($P < 0.05$).

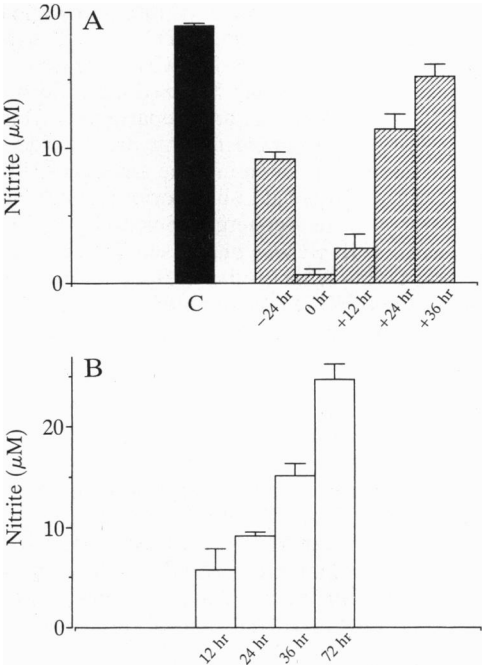

FIG. 3. (*A*) Dependence of inhibition of LPS/IFN-γ-induced nitrite release on exposure to bFGF. RPE cells were treated with LPS at 1 μg/ml and IFN-γ at 100 units/ml, with (▨) or without (control: C, ■) bFGF at 10 ng/ml added at various times relative to LPS/IFN-γ (added at time 0 hr). For −24 hr, RPE cells were pretreated during 24 hr with bFGF, washed, and reincubated for 72 hr with LPS/IFN-γ. Nitrite production was determined after a total incubation time of 72 hr with LPS/IFN-γ. Values are means ± SEMs for three different experiments, each done in duplicate. (*B*) Dependence of nitrite release on exposure to LPS/IFN-γ. After the stimulation period with LPS at 1 μg/ml and IFN-γ at 100 units/ml for 12, 24, 36, or 72 hr, the medium was recovered, and nitrite content was assayed. Fresh medium without LPS/IFN-γ was added for the remainder of the 72 hr. At the end of the 72 hr this medium was recovered and assayed for nitrite content. Results are the sum of nitrite released during the stimulation period and during the rest of the incubation period. Values are means ± SEMs for three different experiments, each done in duplicate.

nitrite that had accumulated during this time in the culture medium (Fig. 4). A close correlation between nitrite and citrulline production can be seen. As mentioned above, untreated controls (bar 1) released very low levels of the two products, and LPS/IFN-γ (bar 2) induced nitrite and citrulline syntheses by RPE cells. In this case, the proliferation of RPE cells was markedly reduced compared with the control in the absence of LPS/IFN-γ, suggesting that the NO-generating pathway induced in RPE cells may exert a strong cytostatic effect on the cell culture. In agreement with this hypothesis, addition of a potent inhibitor of NOS (bar 3), N^G-monomethyl-L-arginine, which inhibited nitrite and citrulline syntheses by RPE cells, restored normal proliferation of the cultured cells. As a control, the effect of a sustained release of NO on RPE cell culture was also investigated, using a NO donor, 3-morpholinosydnonimine (SIN-1), which spontaneously released NO and *N*-morpholinoimmunoacetonitryle (SIN-1C). The addition of SIN-1 to the culture medium inhibited RPE cell proliferation (Table 2). $NaNO_2^-$ at neutral pH and SIN-1C exhibited no inhibitory effect on cell proliferation, and coincubation of hemoglobin with SIN-1 abolished the effect of SIN-1 (Table 2), confirming that NO and not another breakdown product of SIN-1 was responsible for the antiproliferative effect. An additional correlation between NO production and growth inhibition was provided by the data obtained with bFGF. Fig. 4 shows that bFGF

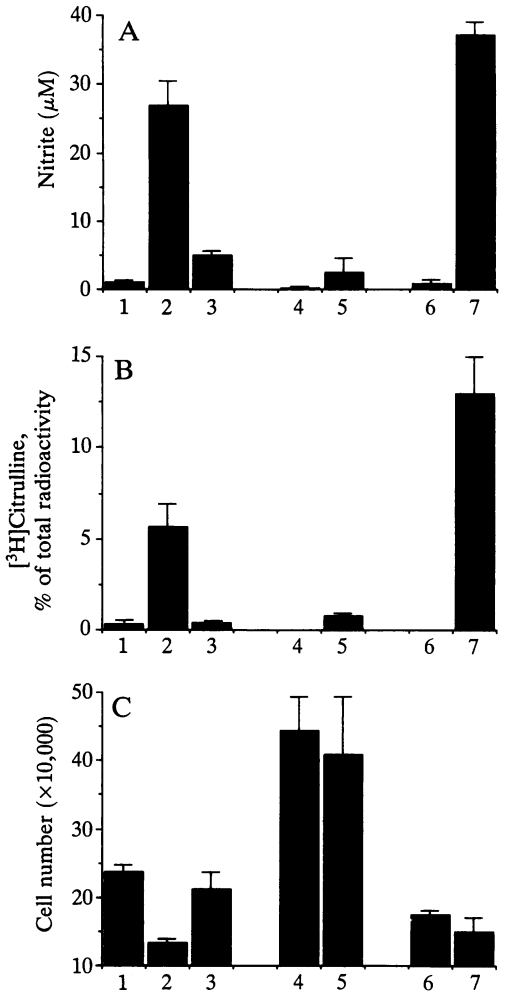

FIG. 4. Correlation between nitrite production, citrulline formation, and antiproliferative effect of LPS/IFN-γ. Subconfluent cells were incubated for 72 hr in fresh DMEM/10% fetal calf serum alone (bar 1) or with LPS at 1 μg/ml plus IFN-γ at 100 units/ml (bar 2), LPS/IFN-γ plus 1 mM N^G-monomethyl-L-arginine (bar 3), bFGF at 10 ng/ml (bar 4), LPS/IFN-γ plus bFGF (bar 5), TGF-β at 10 ng/ml (bar 6), or LPS/IFN-γ plus TGF-β (bar 7). (*A*) Nitrite release was determined in culture supernatant, as described. (*B*) Formation of L-[^{3}H]citrulline was determined in different cell extracts, as described. For bar 3, 0.1 mM of N^G-monomethyl-L-arginine was added in cell extracts. (*C*) Cell proliferation was estimated by counting, as described. Initial cell number was 10^5 cells per well. Values are means ± SEMs for three different experiments, each done in duplicate.

could not induce nitrite and citrulline synthesis and was a potent stimulator of the proliferation of RPE cells *in vitro*: it enhanced cell proliferation 2-fold compared with the control (serum alone). Fig. 4 confirms that bFGF inhibited nitrite release induced by LPS and IFN-γ but also inhibited citrulline formation in the cytosol extracts. When bFGF was added simultaneously with LPS/IFN-γ (bar 5), cellular proliferation was the same as with bFGF alone (bar 4), suggesting that inhibition of the NOS activity by bFGF prevented the antiproliferative effect of LPS/IFN-γ on RPE cells (Fig. 4*C*). With regard to TGF-β, we show that it slightly decreases cell proliferation: 23.5 ± 1.5% inhibition (bar 6) compared with the proliferation induced by serum alone (bar 1). As mentioned above, TGF-β cannot induce NOS activity by itself because neither nitrite production in the culture medium (Fig. 4*A*, bar 6) nor citrulline synthesis by cellular extracts (Fig. 4*B*, bar 6) was detected when RPE cells were cultured for 72 hr with TGF-β at 10 ng/ml. However, when TGF-β was added with LPS/IFN-γ (bar 7), it potentiated the LPS/IFN-γ-induced nitrite release (Fig. 4*A*), as in Fig. 2, and the LPS/IFN-γ-induced citrulline formation (Fig. 4*B*), whereas TGF-β did not significantly modify the antiproliferative effect of LPS/IFN-γ (Fig. 4*C*). In this case, the slight increase of NO due to TGF-β in addition to LPS/IFN-γ does not appear sufficient to increase the important antiproliferative activity from LPS/IFN-γ.

Table 2. Effect of SIN-1 on RPE cell proliferation

	Cell number per dish (× 10^{-4})	
	24 hr	48 hr
Control	6.70 ± 0.10	9.35 ± 0.05
SIN-1 (0.1 mM)	5.95 ± 0.08*	8.40 ± 0.20*
SIN-1 (0.5 mM)	5.05 ± 0.15*	7.70 ± 0.45*
SIN-1C (0.5 mM)	6.90 ± 0.01	9.57 ± 0.60
SIN-1 (0.5 mM) + Hb (200 μM)	6.45 ± 0.01	9.55 ± 0.15
$NaNO_2^-$ (0.5 mM)	6.20 ± 0.30	9.20 ± 0.30

RPE cells were incubated in fresh DMEM/10% fetal calf serum, without (control) or with SIN-1, SIN-1C, $NaNO_2^-$, or hemoglobin (Hb), as indicated. Cells were counted after 24- or 48-hr incubation. Initial cell number was 5 × 10^5 cells per dish. Values are means ± SEMs for three different experiments, each done in duplicate.
*$P < 0.01$, significantly different from control.

DISCUSSION

Our results confirm that treatment of cultured pigmented epithelial cells from bovine retina with LPS and IFN-γ induces NOS activity, as demonstrated by nitrite release (17) and the conversion of L-arginine to L-citrulline in cytosol extracts. Moreover, two members of the FGF family (aFGF and bFGF) inhibit, whereas TGF-β increases, the NO synthesis induced by LPS and IFN-γ in RPE cells. The regulation of NOS activity by growth factors was originally described in macrophages with TGF-β (20) and was also observed in mesangial and vascular smooth muscle, where induction of NOS was markedly inhibited by TGF-β (7, 20, 31). In contrast, our observations show that TGF-β can potentiate the NOS activity (increases of nitrite release in culture medium and citrulline formation by cell extracts) induced by LPS/IFN-γ or by TNF-α/IFN-γ. The positive regulation of NOS activity by TGF-β, described here, adds to the complexity of the effects of TGF-β (32), similar to its contrasting effects on cell proliferation (stimulation or inhibition), and may be correlated with a different expression of its receptor subtypes in various cells (32).

The simultaneous treatment of RPE cells with IFN-γ/LPS and aFGF or bFGF inhibited the nitrite and citrulline production caused by LPS/IFN-γ, reflecting inhibition of NOS activity. An increase in cell proliferation, as is induced by growth factors, is not *per se* sufficient to desensitize cells to induction of NOS by LPS/IFN-γ because EGF, a potent mitogen of RPE cells (23), had no effect on NOS induction. Indeed, we have observed that bFGF and EGF enhanced RPE cell proliferation, respectively, 2- and 1.7-fold compared with serum alone, after 3 days of culture, whereas only bFGF inhibited LPS/IFN-γ-induced NO synthesis. We have attempted to clarify the cellular processes involved in the inhibition of NOS activity by FGFs. In this context, the ability of suramin and tyrphostin to attenuate the inhibitory effect of bFGF suggests the involvement of the high-affinity receptor for FGF, which displays a tyrosine kinase activity, in bFGF-induced inhibition of NOS activity. The potentiation of aFGF-induced NOS inhibition by heparin reinforces this hypothesis. Thus, heparin can substitute for these cell-surface proteoglycans, stabilizing the aFGF, facilitating its access to the high-affinity receptor, and potentiating its inhibitory effect (26, 27). It is not clear by which intracellular

mechanism FGF suppresses LPS/IFN-γ-evoked NO release. Whether the inhibition of the NO production is due to modulation of the NOS gene expression by FGF or whether FGF directly regulates the NOS activity, for instance via a phosphorylation pathway, is not obvious from the present results. A transcriptional regulation seems more likely in view of kinetics results obtained with bFGF. Indeed, we have demonstrated here that continued stimulation of RPE cells with LPS/IFN-γ was essential for induction and maintenance of NO synthesis and that maximal inhibition by FGF was obtained when the growth factor is present during stimulation with LPS/IFN-γ. We thus suggest that inhibition of NOS activity depends on the presence of bFGF during induction, which agrees with the inhibitory effect of TGF-β on NOS activity (7, 20, 31).

In the present report, we further showed that inhibition of RPE cell growth by LPS plus IFN-γ is controlled through the inducible, L-arginine-dependent pathway generating nitrite and citrulline. This conclusion is supported by the correlation established between the amounts of nitrite or citrulline produced in response to different stimuli and the level of cellular proliferation. The antiproliferative action directly depended on NO production, either in RPE cells activated by LPS/IFN-γ or due to the addition of an exogenous source of NO (sydnonimides). It is also noteworthy that inhibition of NOS induction in RPE cells by FGFs correlates with a maintenance of normal cell proliferation. The observations reported here provide further evidence for the inhibition of cell growth by a sustained NO release through the inducible isoform of NOS (11, 25). The different mechanisms, which have been proposed to explain the antiproliferative activity of NO (1, 11), may also account for the antiproliferative effect in the RPE cells, including inhibition of ribonucleotide reductase activity, a radical enzyme essential for DNA synthesis (25, 33).

Formation of NO has been shown to be an important cytotoxic mechanism of activated macrophages (11) and endothelial cells (1, 12). Therefore, NO secreted from RPE cells may act as a cytotoxic compound for microbes or parasites, but NO may also damage healthy cells—namely, the neural retina (photoreceptors and other neural cells) and Müller cells. This cytotoxic property could be an important pathogenic mechanism for the development of different retinal pathologies. FGF, which is present in the interphotoreceptor matrix (34) and in RPE cells themselves (21–23), may prevent the induction of NOS *in vivo*, when RPE cells are stimulated by LPS, TNF-α and IFN-γ, which can be found in the retina, in some pathological situations (19). Our data suggest that bFGF, by inhibiting the inducible isoform of NOS in RPE cells, may have beneficial effects, in that it may protect the retina from damage resulting from cytokine-mediated immunological and inflammatory reactions. Therefore, inhibition of NOS activity may explain some of the protective effects of bFGF in the retina (21, 35) and thus may provide additional prospects in the treatment of retinal inflammatory disorders.

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