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ARTICLES

Inhibition of Endothelial Cell Proliferation and bFGF-Induced Phenotypic Modulation by Nitric Oxide

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Abstract S-nitroso-N-acetyl-D,L-acetylpenicillamine (SNAP), a chemical donor of NO, inhibited serum- and basic fibroblast growth factor (bFGF)-stimulated cultured endothelial cell (EC) proliferation in a dose-dependent manner. The inhibitory effect of NO was reversible after washoff of SNAP-containing media. Measurement of nitrate and nitrite in the media of SNAP-treated EC indicated that decomposition of SNAP into NO reached a stable level at or before 24 h; proliferation of EC was significantly inhibited for another 48 h and recovered thereafter if no additional SNAP was added. The level of NO produced by inhibitory concentrations of SNAP was comparable to NO levels produced by the induction of inducible nitric oxide synthase (iNOS) in smooth muscle cells or retinal pigmented epithelial cells. The growth-inhibitory effect of NO was unlikely to be due to cytotoxicity since 1) cells never completely lost their proliferative capacity even after 10 days of exposure to repeated additions of SNAP, 2) the inhibitory effect was reversible upon removal of NO and with the passage of time, and 3) NO did not reduce the number of cells that were growth-arrested with TGF-β1. In addition to its mitogenic effect, bFGF induced pronounced phenotypic changes, including suppression of contact inhibition, altered cell morphology, and scattering of the cells, in BPAEC cultures, whereas cells treated simultaneously with bFGF and NO did not exhibit these changes. These observations suggest that NO contributes to the regulation of angiogenesis and reendothelialization, processes that require EC proliferation, migration, and differentiation.

Key words: angiogenesis, contact inhibition, inhibitor, SNAP

In addition to being the endothelium-derived relaxing factor, NO is also being recognized as an antiproliferative and cytotoxic agent for many cell types [Blanco et al., 1995; Cornwell et al., 1994; Dawson et al., 1993; Garg et al., 1992; Kroncke et al., 1993; Maragos et al., 1993; Yang et al., 1994]. Situations in which high and sustained amounts of NO may be produced by the induction of iNOS include tumor growth, inflammation, wound healing, and sepsis, in which iNOS-inducing cytokines and bacterial endotoxin are released [for a review see Moncada and Higgs, 1993]. These situations also involve angiogenesis and reendothelialization, the only situations in which EC proliferation occurs in the adult organism [Engerman, 1967; Hobson and Denekamp, 1984; Folkman and Shing, 1992; Klagsbrun and D'Amore, 1991]. Although a number of investigators have reported that NO can regulate EC proliferation and angiogenesis, the effects observed are inconsistent. Using sodium nitroprusside (NaNP) as a chemical NO-donor, Yang et al. [1994] reported NO to be antiproliferative to human umbilical vein EC and bovine aortic EC; Sarkar et al. [1995] reported that NO generated by a variety of chemical donors is antiproliferative to cultured canine EC. Pipili-Synetos et al. [1994] found NO to be antiangiogenic in vivo and in vitro. Another report, however, found the same chemical to stimulate coronary venular EC proliferation and potentiate substance P-induced angiogenesis [Ziche et al., 1994]. Potential drawbacks to using NaNP are that it also releases toxic cyanide (CN^-) and requires redox activation to produce NO, raising the possibility that effects seen due to NaNP addition may be a superimposition of effects produced by NO, CN^-, oxidants, and antioxidants and as such may vary depending on growth conditions and cell type. To clarify the role of NO in EC proliferation and morphogenetic behavior, we investigated modifications in

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EC proliferation by SNAP, which spontaneously produces NO in physiologic buffers. The non-nitroso-containing chemical analog of SNAP, thioacetylpenicillamine (TAP), the other product released when SNAP generates NO, was used as a control to determine whether effects resulting from SNAP treatment were specifically due to NO. In addition, we assayed the level and rate of NO production by SNAP during the incubation as well as the effect of NO on bFGF-induced EC proliferation, neither of which studies were explored by Yang et al. or Sarkar et al. The results showed that NO inhibited serum- and bFGF-induced cultured EC proliferation, and abrogated bFGF-induced phenotypic modifications of EC that may be critical to angiogenesis. These effects of NO are likely due to cytostasis and not cytotoxicity, since the inhibition was reversible, and NO did not cause reduction in the number of growth-arrested cells.

MATERIALS AND METHODS

Cell Culture

Bovine pulmonary arterial endothelial cells (BPAEC) (ATCC, Rockville, MD) were generally grown in RPMI (Sigma, St. Louis, MO) medium containing 10% fetal calf serum (FCS, Life Technologies, Gaithersburg, MD) and supplemented with glutamine, penicillin, and streptomycin. In experiments where nitrate and nitrite concentrations were assayed, RPMI, which contains a high level of nitrate salts, was replaced with DMEM. Bovine pulmonary microvascular endothelial cells (BPMVEC), isolated as described earlier [Del Vecchio et al., 1992] were grown in DMEM (Sigma) containing 10% FCS and supplemented as above. All cell cultures were grown at 37°C in 5% CO_2. Cells were passaged at confluence using trypsin-EDTA (Life Technologies) and replated generally at 1:3 dilution. Cells were occasionally tested for the uptake of Ac-LDL for confirmation of endothelial characteristics.

Cell Proliferation Assay

Cells were plated at a density of 10,000–20,000 per cm^2 in wells of 24-well tissue culture plates. Next day, unattached cells were washed off, the start cell number (T_0) determined from one well, and cells refed with fresh growth media containing the test substance(s) in triplicate or quadruplicate wells and incubated for 3 days under standard growth conditions. Cells were then trypsinized and counted electronically in a Coulter counter Z1 (Coulter Corp., Hialeah, FL). Statistical analyses were performed by the In-Stat program (GraphPad, San Diego, CA) and $P < 0.05$ taken as significant. The MTS-formazan assay for cell proliferation was conducted as follows: cells were plated in a 96-well plate at a density of 20,000/cm^2. SNAP (200 and 500 μM) was added to six wells the next day. After 5 days, 100 μl of media containing the MTS-PMS mix (a gift from Promega, Madison, WI) was added to them according to the manufacturer's protocol. Absorbance at 450 nm was read 2–3 h after addition of MTS.

Assay of Nitrate and Nitrite Production

NO in the supernatant was assayed by measuring nitrate and nitrite (NOx^- for short) concentration by the Griess reaction using a nitrate/nitrite assay kit purchased from Alexis Corp (San Diego, CA). This reaction is specific for the nitrite ion, and endproduct of NO metabolism. Aliquots (usually 80 μl) of filtered or centrifuged conditioned cell medium (grown in serum-containing DMEM) collected from SNAP-treated and control cells were placed in a 96-well plate. Nitrate was reduced to nitrite using nitrate reductase according to the manufacturer's protocol. Then 50 μl each of Griess Reagent I (sulfanilamide) and Griess Reagent II (N-1-naphthyl-ethylenediamine) were added to the supernatants and the mixture incubated at room temperature for 10–15 min. Absorbance at 550 nm was read by a Dynaphot MR-600 microplate reader. To determine nitrite concentration as well as whether all the nitrate was reduced, absorbance values were compared with standard curves drawn with sodium nitrate and sodium nitrite.

Pharmacologic Agents

The NO donor SNAP (Calbiochem) was dissolved at a concentration of 0.05 M in ethanol. TAP, chemically synthesized, was also dissolved in ethanol at a concentration of 0.1 M. Additional ethanol was added to control wells whenever necessary to equalize the amount of ethanol in control and experimental wells. Human recombinant bFGF and TGF-β1 were purchased from R & D Systems (Minneapolis, MN) and reconstituted in 0.1% BSA-containing PBS.

RESULTS

The NO Donor, SNAP, Inhibits Endothelial Cell Proliferation

Exposure of BPAEC to SNAP resulted in a dose-dependent inhibition of serum-induced cell

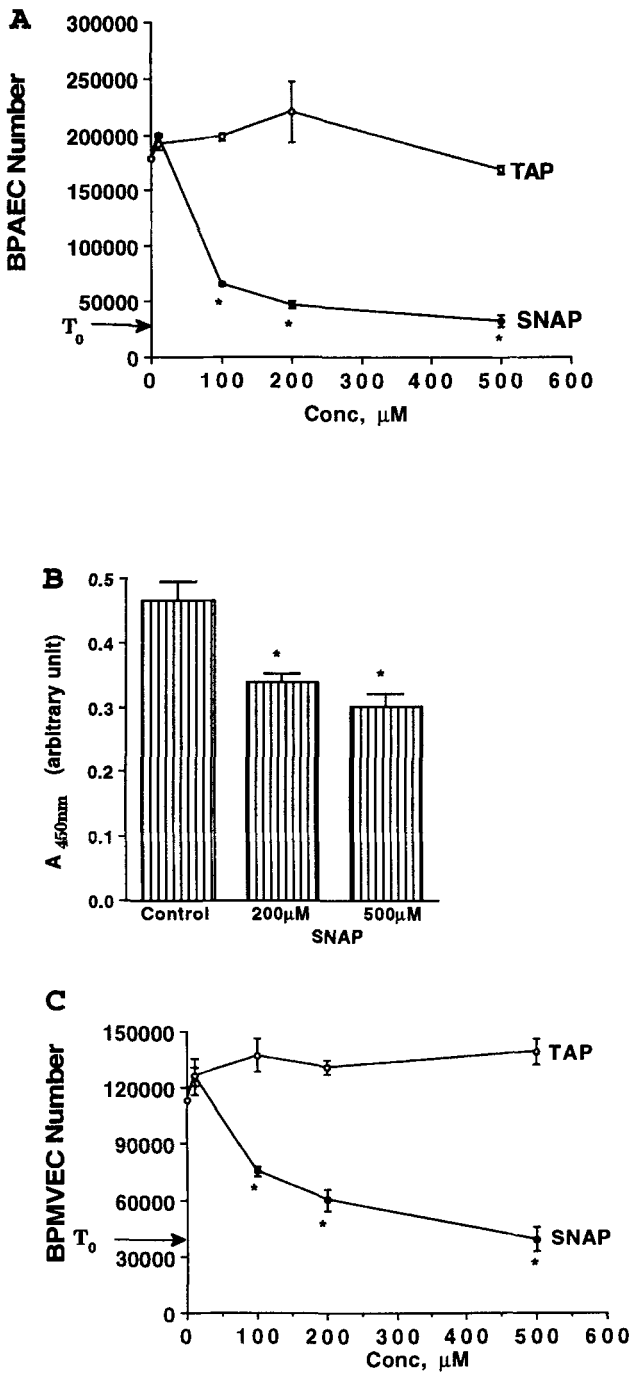

Fig. 1. Inhibition of BPAEC proliferation by NO as determined by (**A**) cell count and (**B**) Metabolic Assay. (**C**) Inhibition of BPMVEC proliferation by NO as determined by cell count. TAP = Thioacetylpenicillamine. T_0 = Cell number at start. A and C were 3-day assays; B was a 5-day assay. 100–500 μM SNAP treatments produced significantly different cell numbers and absorbance. The less dramatic difference between control and SNAP treatment in B is probably due to the longer time of incubation, which cause contact inhibition of control cells and partial recovery in SNAP-treated cells. A and C represent one assay from three similar experiments. Error bars represent ± SD. *Indicates statistically significant difference from control ($P < 0.05$).

TABLE I. Nitric Oxide Metabolites From SNAP-Treated Conditioned Media

[SNAP], μM	[Nitrate + Nitrite], μM
0	low (< < 1 μM)
100	30–35
200	60–70
500	150
1000	> 200

growth (Fig. 1A). A second cell proliferation assay, based on the conversion of MTS into formazan by mitochondrial dehydrogenases present only in viable cells, confirmed the inhibitory effect of SNAP as well as the viability of SNAP-treated cells even after 5 days of exposure (Fig. 1B). TAP, the non-nitroso-containing chemical analogue of SNAP, had no significant effect on BPAEC or on BPMVEC, which underwent a similar growth-inhibition in response to SNAP (Fig. 1C). In addition to these bovine pulmonary EC, SNAP inhibited growth of human pulmonary arterial and dermal EC, rat brain EC, and an immortalized mouse brain microvascular endothelial cell line bEND.3, indicating that this effect is not species- or tissue-specific (data not shown). Measurement of NOx^- concentration by the Griess reaction to determine the amount of NO released by SNAP showed that 100 μM SNAP, the lowest dose at which significant growth inhibition was observed, released 30–35 μM NOx^- in the supernatant; higher concentrations of SNAP released proportionately higher NOx^- levels (Table I). The proliferation of these EC in the presence of NOS-inhibitors L-NAME and L-NIO, however, was not significantly affected, indicating endogenous NO produced by unstimulated EC is not present in sufficiently high quantities to act as an autocrine growth regulator (not shown). Indeed, determination of nitrite/nitrate levels from the conditioned media of unstimulated cells showed much lower NO concentrations ($< < 1$ μM) than those released by antiproliferative concentrations of SNAP.

EC exposed to SNAP for up to 10 days showed no obvious signs of toxic exposure, and did not internalize trypan blue. Cells incubated with SNAP for 3 days resumed significant growth after SNAP was washed out and fresh culture media added. As control cells underwent contact inhibition, cells from which SNAP had been washed off ultimately reached same cell numbers as untreated controls, whereas cells to which SNAP was readded had significantly lower cell numbers (Fig. 2).

In another experiment BPAEC were growth-arrested by treating them with TGF-β1 (5 ng/ml) for 48 h; then one set of samples was exposed to 200 μM SNAP and fresh TGF-β1 for 4 more days while another set was exposed only to fresh TGF-β1. There was no difference seen in the cell number between samples treated with SNAP and TGF-β1 and those treated only with TGF-β1—in both cases, cell numbers remained at the plating number at start (Fig. 3). Since the growth of these cells was completely abolished by TGF-β1, a reduction in cell number in the (SNAP + TGF-β1)-treated samples compared to TGF-β1-treated samples should have occurred if SNAP were cytotoxic. Control experiments showed that if TGF-β1 and SNAP were together added to rapidly-growing cells, they had additive growth-inhibitory effect, demonstrating that TGF-β1 did not protect cells from the SNAP effect (not shown). It is unlikely that the absence of a reduction in cell number is due to insensitivity of nonproliferating cells to cytotoxicity, since similar experiments showed SIN-1, a peroxynitrite donor, to be very toxic on TGF-β-arrested cells [A. RayChaudhury, in press].

To determine the time course of the action of SNAP, BPAEC were incubated with 200 μM SNAP and cell counts were made after 1 day, 3 days, and 6 days of incubation without supply of additional SNAP. As shown in Figure 4, after only 1 day (~ 24 h) of SNAP treatment a significant level of growth inhibition was effected by SNAP and about 60 μM NO as assayed by the Griess reaction was produced. Over the next 2 days SNAP-treated cells grew much more slowly than untreated cells even though no additional NO production was detected. Between Day 3 and Day 6, proliferation of untreated cells slowed down considerably, most likely due to a combination of serum depletion and increased contact inhibition. SNAP-treated cells during this period proliferated slightly faster than control cells, indicating that they were recovering from NO. These observations suggest 1) that NO release from SNAP reaches a maximum in ≤ 24 h, and 2) the antiproliferative effect of NO persists for at least 48 h after cells have been exposed to NO.

Since the effect of NO persisted after all NO was released and since washoff could restore the full growth potential of the EC, we investigated whether NO was inducing an autocrine secreted growth inhibitor from EC. However, CM col-

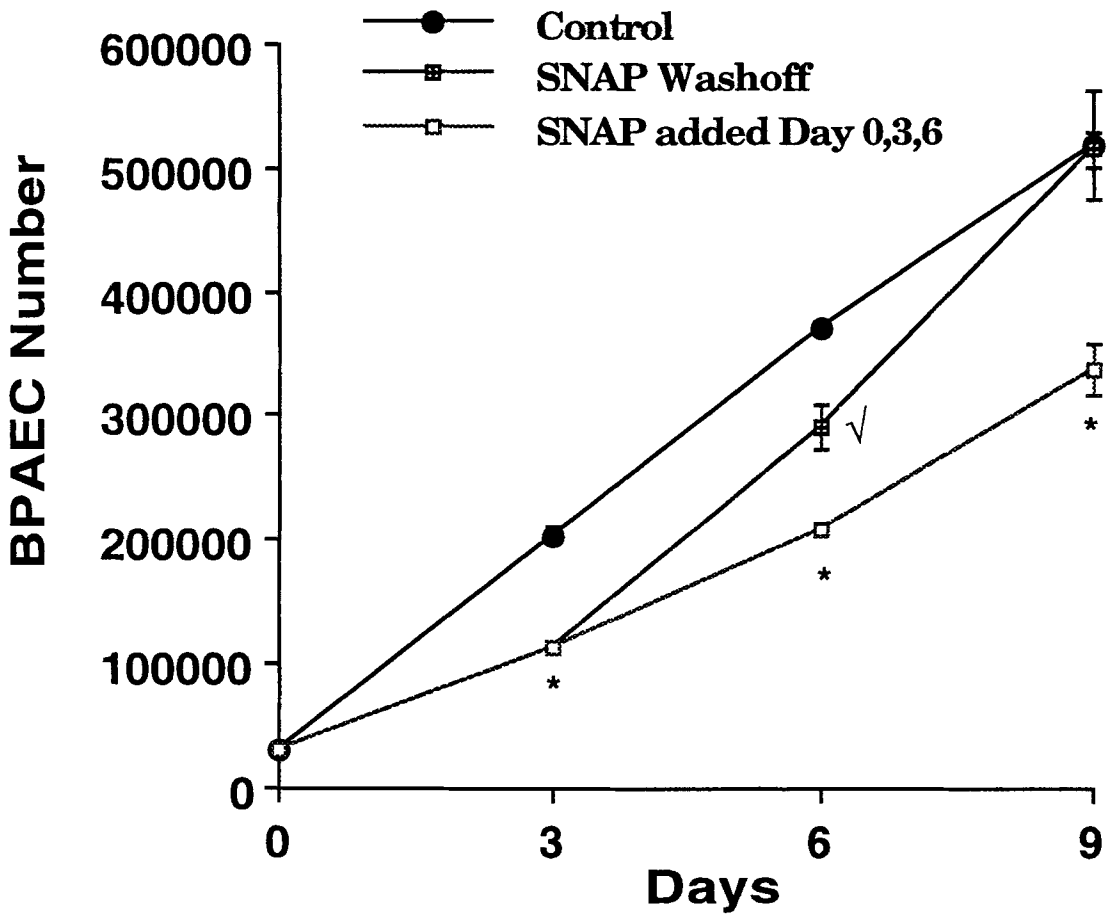

Fig. 2. Reversibility of NO-induced inhibition of BPAEC proliferation. SNAP was used at a concentration of 200 μM, and in one set added on Day 0, 3, and 6 and in another set washed off on Day 3. Control cells received ethanol at equal volume. Data show one assay of three similar experiments. * and √ indicate statistically significant difference from control.

lected from SNAP-treated cells applied to fresh BPAEC at a 50% concentration (supplemented with 50% regular growth medium to compensate for spent serum) did not have any inhibitory effect (data not shown).

NO Inhibits Stimulation of EC Proliferation by bFGF

Our previous experiments demonstrated that NO inhibits the serum-induced proliferation of EC. To determine whether NO also inhibits the stimulation of EC proliferation by bFGF, a potent EC mitogen and angiogenic agent, we treated BPAEC with 1) 10% serum alone, 2) 10% serum + bFGF (5 and 25 ng/ml), 3) 10% serum + SNAP (200 and 500 μM) and 4) 10% serum + bFGF + SNAP. As shown in Figure 5, bFGF exerts a two-fold stimulation over the serum-stimulated proliferation of BPAEC. However, in the presence of 500 μM SNAP, there is no difference in cell numbers between cells treated with bFGF and those untreated with bFGF, showing NO can completely block the mitogenic stimulation by bFGF. Control studies showed that 500 μM SNAP produced about 2.5-fold as much NO as 200 μM SNAP.

NO Inhibits bFGF-Induced Phenotypic Changes in EC

In the presence of bFGF (5–25 ng/ml), the BPAEC monolayer was completely disrupted, cells were scattered, grew in an overlapped pattern, and underwent morphologic changes. In addition, bFGF-treated cells detached readily on gentle washing with PBS. Cells treated with both bFGF and 500 μM SNAP, however, retained the contact-inhibited growth pattern, remained firmly attached to the substratum and showed only slight morphologic changes (Fig. 6).

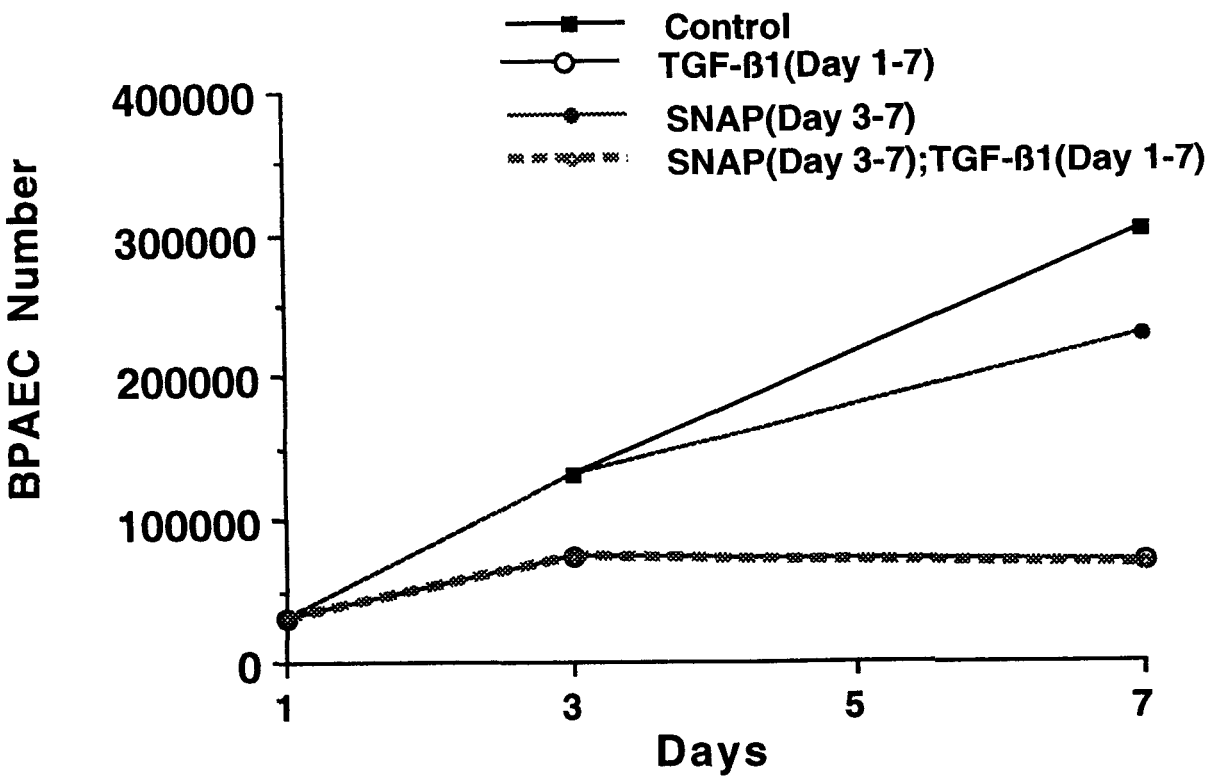

Fig. 3. NO does not cause reduction in the number of growth-arrested cells. BPAEC were growth arrested with 5 ng/ml of TGF-β1 for 2 days (Day 1–3). SNAP (200 μM) was added to one set of wells together with 5 ng/ml fresh TGF-β1, in an equal number of wells only fresh TGF-β1 was added. Whereas SNAP inhibited growth of non-TGF-β-treated cells ($P < 0.05$), it did not reduce the number of TGF-β-treated growth arrested cells, indicating SNAP is not killing cells.

DISCUSSION

Proliferation of EC is an essential step for angiogenesis—in the normal adult, EC have a very low turnover rate whereas in angiogenesis-dependent processes, such as reproduction and tumor metastasis, EC actively proliferate. This study identifies NO as a potent inhibitor of EC proliferation. Our results are consistent with the reports of Yang et al. [1994] and Sarkar et al. [1995]; in addition, we have convincing evidence for the reversible and nontoxic nature of this inhibition.

The lowest concentration of NO that incurred near-maximal growth inhibition was 30–35 μM. This level is comparable to NO released by smooth muscle cells (SMC) or retinal pigmented epithelial cells on stimulation with cytokines or lipopolysaccharide [Fukuo et al., 1995; Goureau et al., 1992], indicating that EC may indeed be exposed to paracrine antiproliferative doses of NO in the vasculature. Direct measurements of NO in vivo have been rare due to experimental difficulties and the short half-life of NO; however, Malinski et al. [1993], using a microsensor, reported NO concentrations of up to 4 μM in rat brain tissue in vivo after transient cerebral artery occlusion. Thus, there is a reasonable likelihood that in pathologic situations (such as inflammation and sepsis) where iNOS may be turned on, antiproliferative concentrations of NO will be generated in the endothelial microenvironment.

The effect of NO can persist well after cells have been exposed. However, although cells eventually recovered from the antiproliferative effect of NO in the absence of further addition, we did not see any indication of BPAEC getting resistant to NO-induced growth-inhibition—if fresh NO was added, growth inhibition was maintained. A study by Fukuo et al. [1995] found that whereas NO alone (as derived from NaNP) inhibited EC proliferation, treatment of EC-SMC cocultures with NaNP as well as CM from NaNP-treated SMC stimulated DNA synthesis in EC. This stimulatory activity was partially attributed to bFGF being released due to the cytotoxic effect of NO on SMC. Our results and those of Fukuo et al. are not inconsistent, since

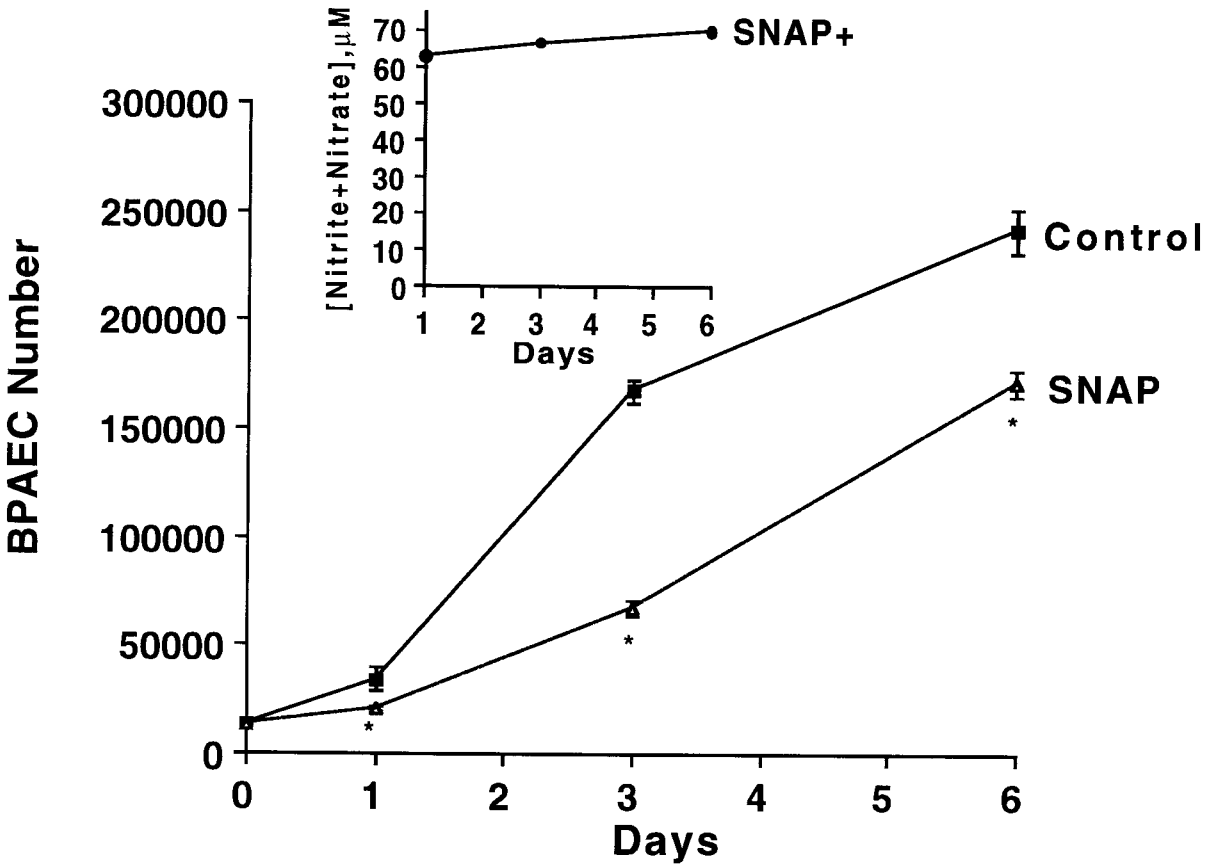

Fig. 4. NO release from SNAP is complete in 24 h; EC proliferation is inhibited for at least 72 h but recovers. NO release from SNAP was assessed by the Griess reaction. *Denotes statistically significant difference from control.

whereas in our studies SNAP and bFGF were added simultaneously, it is doubtful whether in their studies EC were being acted on by both NO and bFGF during the assays. As we have shown, single exposure to NO inhibits EC proliferation for up to 72 h but cells recover proliferative ability thereafter; in their assays cultures were treated with NaNP (or IL-1) for 72 h and then thymidine assays were conducted for 24 h. If SMC were lethally injured by autocrine NO, releasing bFGF, EC were exposed to a short burst of paracrine NO, and then continually to bFGF. During the thymidine assay (96 h post-NaNP addition), EC may have recovered from the antiproliferative effect of NO but still exposed to bFGF. Further, the blocking effect of NO on bFGF-induced proliferation was dose-dependent—whereas 200 μM SNAP had a partial effect, 500 μM SNAP completely inhibited bFGF-induced mitogenesis. In situations like inflammation and tumorigenesis, EC may be continually exposed to NO from SMC, macrophages, and neutrophils (in addition to endogenous NO), consequently, the antiproliferative effect of NO may be more pronounced and prolonged.

bFGF induces matrix-degrading protease activity in EC [Pepper et al., 1990; Saksela et al., 1987], an effect that may play a role in bFGF-induced EC proliferation, migration, and angiogenesis. The phenotypic changes observed in BPAEC after bFGF-treatment—scattered distribution of cells, abrogation of contact inhibition, and easy detachability may arise from a combination of the chemokinetic and protease-activating actions of bFGF, resulting in degradation of the subendothelial matrix and allowing freer movement of cells. The mitogenic and phenotypic changes may be related—TIMP-2, an inhibitor of matrix-degrading metalloproteinases, abolishes bFGF-induced proliferation of EC [Murphy et al., 1993]. NO reversed the scattered growth pattern of bFGF-treated cells, as well as their easy detachability, indicating that NO may strengthen cell-cell and/or cell-matrix adhesion and may at least in part exert its antiprolifera-

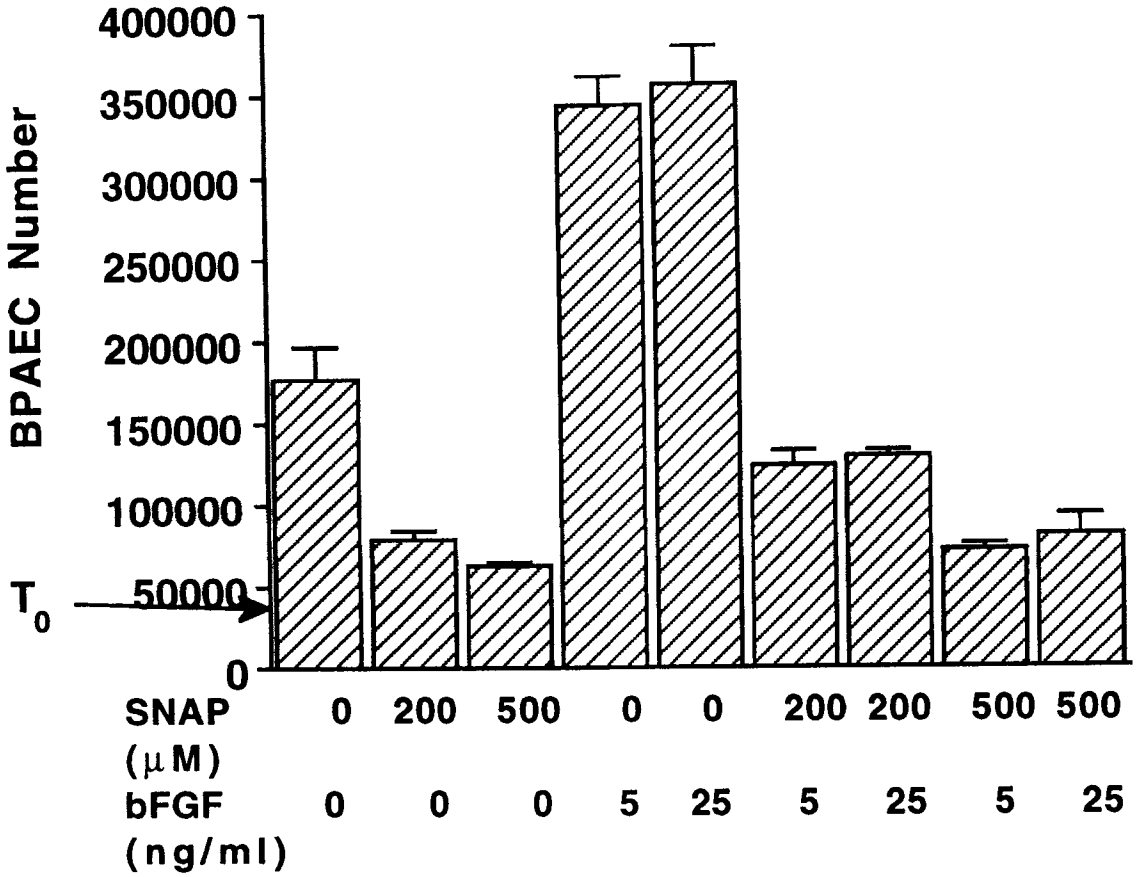

Fig. 5. NO inhibits bFGF-induced EC proliferation. SNAP and bFGF were added simultaneously at the concentrations indicated. Assay was conducted over 3 days. Data show one assay of three similar experiments.

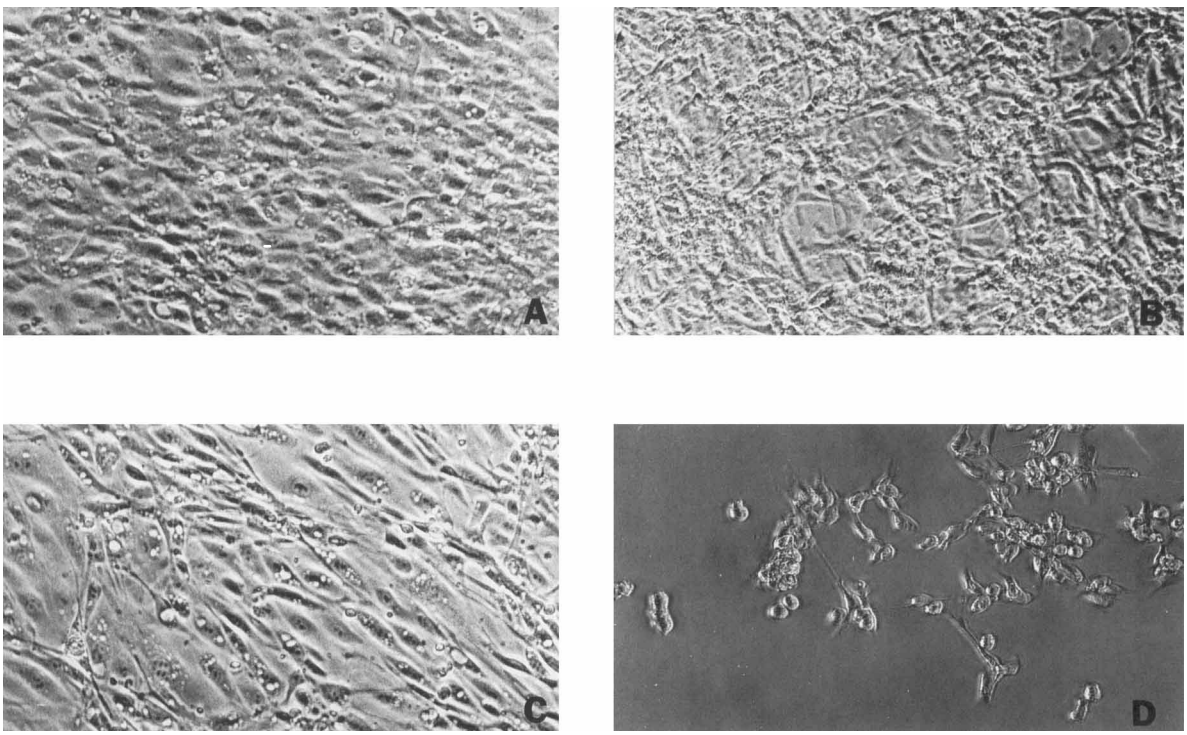

Fig. 6. NO reverses bFGF-induced phenotypic changes in BPAEC. Phase-contrast photographs of (A) control BPAEC, (B) 10 ng/ml bFGF-treated, (C) simultaneously 10 ng/ml bFGF- and 500 μM SNAP-treated. (D) bFGF-treated cells after gentle washing (2 × PBS). Treatment time was 3 days; magnification 10×.

tive effect through such a mechanism. Indeed, Pellegata et al. [1994] have reported that NOS activity upregulates fibronectin production by HUVEC.

The reversal of bFGF-induced morphologic and phenotypic changes by NO is particularly intriguing since these changes (loss of contact inhibition, altered morphology, scattering) may explain how bFGF incurs EC migration and proliferation in an contact-inhibited monolayer during angiogenesis. bFGF has been implicated as an angiogenic agent in several tumors and other angiogenesis-dependent diseases [Folkman and Shing, 1992; Schultz-Hector and Haghayegh, 1993; Takahashi et al., 1991] and is responsible for EC proliferation following vascular injury [Lindner et al., 1990]. A number of physiologic antiangiogenic agents have been identified, such as thrombospondin-1 [Good et al., 1990] and angiostatin [O'Reilly et al., 1994], which by preventing neovascularization in the normal adult organism may act as a defense mechanism against angiogenesis-dependent diseases. NO may serve a similar purpose in vivo. bFGF attenuates the level of iNOS expression [Goureau et al., 1993] which may contribute to its angiogenic potential. Another angiogenesis-dependent disease, diabetic retinopathy, generates advanced glycosylation endproducts which can quench NO and reverse the antiproliferative action of NO [Hogan et al., 1992]. Thus, the irregular angiogenesis in many pathologic situations may at least be partially aided by suppression of NO.

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REFERENCES

Blanco FJ, Ochs RL, Schwarz H, Lotz M (1995): Chondrocyte apoptosis induced by nitric oxide. Am J Pathol 146:75–85.

Cornwell TL, Arnold E, Boerth NJ, Lincoln TM (1994): Inhibition of smooth muscle cell growth by nitric oxide and activation of cAMP-dependent protein kinase by cGMP. Am J Physiol 267:C1405–1413.

Dawson VL, Dawson TM, Bartley DA, Uhl GR, Snyder SH (1993): Mechanisms of nitric oxide-mediated neurotoxicity in primary brain cultures. J Neurosci 13:2651–2661.

Del Vecchio PJ, Siflinger-Birnboim A, Belloni PA, Holleran LA, Lum H, Malik AB (1992): Culture and characterization of pulmonary microvascular endothelial cells. In Vitro Cell Dev Biol 28A:711–715.

Engerman RL, Pfaffenbach D, Davis MD (1967): Cell turnover of capillaries. Lab Invest 17:738–743.

Folkman J, Shing Y (1992): Angiogenesis. J Biol Chem 267:10931–10934.

Fukuo K, Inoue T, Morimoto S, Nakahashi T, Yasuda O, Kitano S, Sasada R, Ogihara T (1995): Nitric oxide mediates cytotoxicity and basic fibroblast growth factor release in cultured vascular smooth muscle cells. A possible mechanism of neovascularization in atherosclerotic plaques. J Clin Invest 95:669–676.

Garg UC, Devi L, Turndorf H, Goldfrank LR, Bansinath M (1992): Effect of nitric oxide on mitogenesis and proliferation of cerebellar glial cells. Brain Res 592:208–212.

Good DJ, Polverini PJ, Rastinejad F, Le Beau MM, Lemons RS, Frazier WA, Bouck NP (1990): A tumor suppressor-dependent inhibitor of angiogenesis is immunologically and functionally indistinguishable from a fragment of thrombospondin. PNAS 87:6624–6628.

Goureau O, Lepoivre M, Becquet F, Courtois Y (1993): Differential regulation of inducible nitric oxide synthase by fibroblast growth factors and transforming growth factor beta in bovine retinal pigmented epithelial cells: Inverse correlation with cellular proliferation. PNAS 90: 4276–4280.

Goureau O, Lepoivre M, Courtois Y (1992): Lipopolysaccharide and cytokines induce a macrophage-type nitric oxide synthase in bovine retinal pigmented epithelial cells. Biochem Biophys Res Commun 186:854–859.

Hobson B, Denekamp J (1984): Endothelial proliferation in tumours and normal tissues: Continuous *labeling* studies. Br J Cancer 49:405–413.

Hogan M, Cerami A, Bucala R (1992): Advanced glycosylation endproducts block the antiproliferative effect of nitric oxide. Role in the vascular and renal complications of diabetes mellitus. J Clin Invest 90:1110–1115.

Klagsbrun M, D'Amore PA (1991): Regulators of angiogenesis. Annu Rev Physiol 53:217–239.

Kroncke KD, Brenner HH, Rodriguez ML, Etzkorn K, Noack EA, Kolb H, Kolb-Bachofen V (1993): Pancreatic islet cells are highly susceptible towards the cytotoxic effects of chemically generated nitric oxide. Biochim Biophys Acta 1182:221–229.

Lindner V, Majack R, Reidy MA (1990): Basic FGF stimulates endothelial regrowth and proliferation in denuded arteries. Effect of bFGF on endothelial recovery following injury. J Clin Invest 85:2004–2008.

Malinski T, Bailey F, Zhang ZG, Chopp M (1993): Nitric oxide measured by porphyrinic microsensor in rat brain after transient middle cerebral artery occlusion. J Cereb Blood Flow Metab 13:355–358.

Maragos CM, Wang JM, Hrabie JA, Oppenheim JJ, Keefer LK (1993): Nitric oxide/nucleophile complexes inhibit the in vitro proliferation of A375 melanoma cells via nitric oxide release. Cancer Res 53:564–568.

Moncada S, Higgs A (1993): The L-arginine-nitric oxide pathway. New Eng J Med 329:2002–2012.

Murphy AN, Unsworth EJ, Stetler-Stevenson WG (1993): Tissue inhibitor of metalloproteinases-2 inhibits bFGF-induced human microvascular cell proliferation. J Cell Physiol 157:351–358.

O'Reilly MS, Holmgren L, Shing Y, Chen C, Rosenthal RA, Moses M, Lane WS, Cao Y, Sage EH, Folkman J (1994): Angiostatin: A novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma. Cell 79:315–328.

Pellegata F, Radaelli A, Ferrero E, Toninelli E, Vidal MJ, Chierchia SL, Zocchi MR (1994): Inducible nitric oxide synthase modulates fibronectin production in the EA.hy926 cell line and cultured human umbilical vein endothelial cells. J Cardiovasc Pharmacol 24:1014–1019.

Pepper MS, Belin D, Montesano R, Orci L, Vassalli J-D (1990): Transforming growth factor-beta1 modulates basic fibroblast growth factor-induced proteolytic and angiogenic properties of endothelial cells in vitro. J Cell Biol 111:743–755.

Pipili-Synetos E, Sakkoula E, Haralabopoulos G, Andriopoulou P, Peristeris P, Maragoudakis ME (1994): Evidence that nitric oxide is an endogenous antiangiogenic mediator. Br J Pharmacol 111:892–900.

Saksela O, Moscatelli D, Rifkin DB (1987): The opposing effects of basic fibroblast growth factor and transforming growth factor beta on the regulation of plasminogen activator activity in capillary endothelial cells. J Cell Biol 105:957–963.

Sarkar R, Webb RC, Stanley JC (1995): Nitric oxide inhibition of endothelial cell mitogenesis and proliferation. Surgery 118:274–279.

Schultz-Hector S, Haghayegh S (1993): β-fibroblast growth factor expression in human and murine squamous cell carcinomas and its relationship to regional endothelial cell proliferation. Cancer Res 53:1444–1449.

Takahashi JA, Fukumoto M, Kozai Y, Ito N, Oda Y, Kikuchi H, Hatanaka M (1991): Inhibition of cell growth and tumorigenesis of human glioblastoma cells by a neutralizing antibody against human basic fibroblast growth factor. FEBS Lett 288:65–71.

Yang W, Ando J, Korenaga R, Toyo-oka T, Kamiya A (1994): Exogenous nitric oxide inhibits proliferation of cultured vascular endothelial cells. Biochem Biophys Res Commun 203:1160–1167.

Ziche M, Morbidelli L, Masini E, Amerini S, Granger HJ, Maggi CA, Geppeti P, Ledda F (1994): Nitric oxide mediates angiogenesis in vivo and endothelial cell growth and migration in vitro promoted by Substance P. J Clin Invest 94:2036–2044.