Effects of Candidate Autocrine and Paracrine Mediators on Growth Responses in Isolated Rat Arteries

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Abstract We evaluated the effects of mediators that can be produced by smooth muscle and endothelial cells on growth responses in isolated arteries. Segments of carotid and renal arteries, denuded of endothelium, were isolated from adult rats and studied during tissue culture in the presence of indomethacin. Three days of culture in the presence of serum stimulated DNA synthesis in the media. During long-term culture new layers of cells developed at the borders of the arterial segments. Medial DNA synthesis depended less on serum than extramedial cell proliferation. During moderate stimulation, basic fibroblast growth factor and endothelin-1 enhanced and interleukin-1 and transforming growth factor-β reduced medial DNA synthesis, whereas insulin-like growth factor-1, platelet-derived growth factor AA, platelet-derived growth factor BB, and angiotensin II were without effect. Of these factors, only endothelin-1 stimulated extramedial cell proliferation. In addition, serum-stimulated but not basic fibroblast growth factor-stimulated medial DNA synthesis was reduced in arteries that had not been denuded of endothelium than in de-endothelialized arteries. Differences between preparations with and without endothelium persisted in the absence of L-arginine and in the presence of an inhibitor of nitric oxide synthase. These observations confirmed that DNA synthesis in the arterial media and extramedial cell proliferation are influenced by different factors. They further indicated that endothelial modulation of medial DNA synthesis does not seem to involve endothelium-derived prostaglandins, nitric oxide, or interleukin-1 and that it can be blunted by basic fibroblast growth factor. (Arterioscler Thromb. 1994;14:420–426.)

Key Words • endothelium • growth factors • DNA synthesis • arterial smooth muscle cell proliferation • arterial tissue culture • transforming growth factor-β • interleukin-1 • basic fibroblast growth factor • angiotensin II • platelet-derived growth factor • endothelin-1 • indomethacin

Mechanisms of arterial smooth muscle cell (ASMC) growth control have been studied primarily by cell culture and by balloon-catheter injury in experimental animals.3-4 These approaches indicate that vascular cells can produce peptide growth factors, cytokines, and vasoactive agents that can interfere with growth responses.5-9 This is not restricted to ASMCs but applies to endothelial cells as well.10-15 A role for autocrine and paracrine mediators in vascular growth responses depends not only on their production but also on the responsiveness of the cell types involved.16 Many agents have been shown to promote proliferation of isolated ASMCs in vitro,14,17-21 but this does not necessarily predict their activity in the vascular wall.22,23 Several causes have been suggested as underlying the different effects of growth factors in isolated and intravascular ASMCs. These include differences in cell density, phenotype, and presence of extracellular matrix components24 and the multifactorial nature of ASMC growth control in situ, including not only growth factors but also growth inhibitors.24-27 In general, analysis of the effects of exogenous agents and contributions of endogenous factors to growth responses in vivo are complicated by the lack of sufficient agonists, the inavailability of selective inhibitors, and the occurrence of both systemic and local responses during drug treatment. Furthermore, influences of the endothelium on the underlying ASMCs28-30 may interfere with structural responses of the vascular wall to administered agents.

In this study, therefore, we evaluated effects of candidate autocrine and paracrine mediators on DNA synthesis and cellular proliferation in isolated arteries during organ culture.31,32 In this experimental system, ASMCs are not dispersed and thus remain at high density and in contact with their natural extracellular matrix; both cell kinetics and changes of arterial structure can be studied; known concentrations and combinations of growth factors can be applied; and the experiments can be performed in the absence and presence of endothelium. The choice of agents, which included peptide growth factors, cytokines, and vasoactive agents, was inspired by their action on ASMC proliferation during cell culture.14,17,21 and by the possibility that they can be delivered to the arterial wall both by the ASMCs themselves3,9 and by endothelial cells.10-15

Methods

The experiments were performed in segments of carotid and renal arteries that had been isolated from 20-week-old male Wistar-Kyoto rats and chemically sympathectomized in vitro with 6-hydroxydopamine (300 µg/mL; Sigma Chemical Co).33,34 Except when specifically mentioned, the segments were mechanically denuded of endothelium by passing the shaft of a hypodermic needle (outer diameter, 0.8 and 0.7 mm diameter) through the arterial wall. The preparations were cultured in Eagle's minimum essential medium with Earle's balanced salt solution (1:1, vol/vol), supplemented with 5% fetal bovine serum, 100 U/mL penicillin, 100 µg/mL streptomycin, and 0.4 µg/mL amphotericin B (Sigma). The segments were cultured at 37°C in an atmosphere of 95% air and 5% CO2 at an initial pressure of 95 cm H2O. Each segment was covered with a filter to prevent convectional outflow of culture fluid. After an initial period of equilibration, the media were replaced with fresh Eagle's minimum essential medium with Earle's balanced salt solution (1:1, vol/vol) containing 2% fetal bovine serum, 100 U/mL penicillin, 100 µg/mL streptomycin, 0.4 µg/mL amphotericin B, and various test agents added fresh to the media. The preparations were cultured continuously at 37°C in an atmosphere of 95% air and 5% CO2 at an initial pressure of 95 cm H2O with the filter in place. The media were changed every 2 days. At the end of the culture period, the preparations were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet. The arterial preparations were studied under an Axioskop microscope (Carl Zeiss, Oberkochen, Germany) equipped with an attached camera (Panasonic, Osaka, Japan) connected to a computer for image analysis (Image-Pro Plus, Media Cybernetics, Silver Spring, Md).
for carotid and renal arteries, respectively) through the arterial lumen. Removal of the endothelium was confirmed by the absence of luminal immunoreactivity for von Willebrand factor and angiotensin-converting enzyme and by the absence of relaxing responses to acetylcholine and histamine. We have previously demonstrated that denudation did not appreciably affect the DNA content of the arterial preparations or their maximal contractile responses to high potassium, indicating that no medial injury was induced.

**Sera and Agents**

Fetal calf serum (FCS), a classic but undefined source of growth factors, appears to contain high concentrations of serotonin and angiotensins (P.M.H. Schiﬀers, May 1993, unpublished observations). For this reason, the serum was dialedyzed (cutoff point, 6 kDa) against three changes of phosphate buffer, pH 7.4, at 4°C for 24 hours. Both serum-derived serum (PDS) and CM Sephadex-PDS (CMS-PDS) were prepared by a method described by Vogel et al. In brief, rat blood anticoagulated with sodium citrate was spun at 3000 rpm for 20 minutes at 4°C. The supernatant was respun at 13 500 rpm for 20 minutes at 4°C to remove the platelets. After the second spin, 1.0 mol/L CaCl2 was added to a ﬁnal concentration of 20 mmol/L, and Krebs-Ringer bicarbonate solution (KRB; composition in mmol/L, NaCl 118.5; KCl 4.7; MgSO4 1.2; KH2PO4 1.2; NaHCO3 25.0; CaCl2; and glucose 11.1) was added in a ratio of 1 to 6 vol plasma. The plasma was then incubated at 37°C for 2 hours to allow clot formation and spun at 13 500 rpm for 30 minutes at 4°C. The supernatant was dialyzed against three changes of 0.1 mol/L tris(hydroxymethyl)aminomethane (Tris)-HCl, pH 7.4, at 4°C for 24 hours. Part of this PDS was then applied to a CM Sephadex column (50x2 cm) and approximately eight times the volume of fluid that was applied to the column was collected by using 0.01 mol/L Tris-HCl/0.09 mol/L NaCl, pH 7.4, as eluants. The eluate was concentrated back to the volume of Griess reagent (0.5% sulfanilamide/0.05% N-[1-naphthyl]ethylenediamine dihydrochloride) and incubated at 56°C for 30 minutes, centrifuged at 3000 rpm at 4°C for 20 minutes, ﬁltered, and stored at −70°C until use.

The homodimers of platelet-derived growth factor (PDGF) and endothelin-1 (ET-1) were obtained from Bachem. Transforming growth factor-β (TGF-β) and basic ﬁbroblast growth factor (bFGF) were purchased from Peninsula Laboratories. Angiotensin II (Ang II) and N^6^-nitro-L-arginine-methyl ester (L-NAME) were obtained from Sigma Chemical Co. L-Arginine–free medium was prepared by Gibco. Insulin-like growth factor-1 (IGF-1) was a kind gift from Dr K. Hofbauer (CIBA-GEIGY). Interleukin-1 (IL-1) was kindly supplied by Dr W. Buurman (University of Limburg, Maastricht, the Netherlands).

**Tissue Culture**

Arterial organoid culture was performed as previously described. All segments were mounted on a 0.25-mm sterile wire (Dental Wire) and individually suspended in 24-well culture dishes (Greiner) ﬁlled with 1 mL Dulbecco’s minimal Eagle’s medium (L-arginine content, 250 μmol/L) containing 2 mmol/L glutamine (GIBCO), 100 IU/ml penicillin (Gist-Brocades), 100 μg/ml streptomycin (Pharmachemie), and 100 ng/mL gentamicin (Pharmachemie). Interleukin-1 (IL-1) was kindly supplied by Prof. H. H. van der Velden (University of Nijmegen, Nijmegen, the Netherlands). Cellular proliferation, the number of cells appearing at the arterial lumen, was counted at the end of culture.

**DNA Synthesis During Short-term Culture**

To estimate DNA synthesis, 1 μmol/L of the thymidine analogue 5-bromo-2'-deoxyuridine (BrDU; Sigma Chemical Co) was included in the culture medium with daily replacement of the medium and label. After 3 days of culture, the arterial segments were rinsed for 60 minutes in drug-free KRB, ﬁxed in paraformaldehyde at 37°C, embedded in paraﬁn, and cut into 4-μm-thick cross sections.

**Results**

**DNA Synthesis During Short-term Culture**

Fig 1 illustrates the extent of nuclear incorporation of BrDU in the media of denuded renal artery segments exposed for 3 days to different media. Signiﬁcant BrDU labeling was already obtained in serum-free medium. The presence of sera further stimulated intra-arterial DNA synthesis. The effect was larger for 20% dFCS

**Short-term Tissue Culture**

To evaluate the effects of exogenous growth factors on medial DNA synthesis, renal arteries were maintained in culture for 4 days in the presence of 20% dFCS, 5% PDS, or 5% CMS-PDS supplemented with either 1 μmol/L Ang II, 20% dFCS.
(10- to 15-fold) than for 5% rat PDS (five- to eightfold). Part of the stimulatory action of PDS could be removed by passage over a Sephadex column (CMS-PDS, two- to fourfold stimulation above that seen in serum-free medium). In denuded carotid artery segments, BrdU labeling of the media was significant after 3 days of incubation in serum-free medium (1.9±0.6%/3 d) and was stimulated 10- to 15-fold by 20% dFCS (12.2±2.5%/3 d). Note that both findings were significantly lower in carotid than in renal arteries (5.2±1.1%/3 d and 38.0±3.6%/3 d in the absence and presence of dFCS, respectively).

Cell Proliferation During Long-term Culture

Culture in the presence of 20% dFCS for up to 2 weeks does not alter the number of cells in the original media of renal or carotid artery segments but gives rise to the formation of new layers of cells outside the original arterial wall. Fig 2 shows that the formation of this periadventitial layer was serum dependent. After 4 days of culture in the presence of 20% dFCS, a small number of cells could already be detected in cross sections of carotid arteries. When at this stage 20% dFCS was removed or replaced by 5% CMS-PDS, the growth of the new layer was prevented. In the continuous presence of 20% dFCS, however, the size of the new layer increased markedly. After 2 weeks of incubation the number of cells in the new layer (987±59/cross section) was markedly larger than that in the original media (321±34/cross section).

Exogenous Growth Factors and Medial DNA Synthesis in Denuded Vessels

Effects of high concentrations of recombinant growth factors on BrdU labeling during mild (CMS-PDS), moderate (PDS), and strong (dFCS) stimulation, and these agents were also tested in combination with IGF-1 (Fig 3).

Exogenous Growth Factors and Medial DNA Synthesis in Denuded Vessels

Effects of high concentrations of recombinant growth factors on BrdU labeling were evaluated during mild (CMS-PDS), moderate (PDS), and strong (dFCS) stimulation, and these agents were also tested in combination with IGF-1 (Fig 3).

In denuded rat renal artery exposed to CMS-PDS, TGF-β and IL-1 reduced BrdU labeling. ET-1 and bFGF significantly stimulated DNA synthesis under these conditions, but PDGF-AA, PDGF-BB, and Ang II were without effect (Fig 3).

By itself IGF-1 moderately and insignificantly enhanced DNA synthesis in CMS-PDS. It did not affect the inhibitory action of TGF-β but abolished the inhibitory action of IL-1 on BrdU labeling of medial cells. Furthermore, IGF-1 unmasked a significant stimulatory effect of PDGF-AA but not PDGF-BB or Ang II. In the presence of IGF-1, ET-1 further increased DNA synthesis (Fig 3).
Effects of the Endothelium

The relatively strong stimulation of DNA synthesis noted in the presence of PDS was reduced by TGF-β and IL-1 and increased by bFGF. It was not affected by PDGF-AA, PDGF-BB, Ang II, or ET-1 (Fig 3).

In the presence of 20% dFCS, which by itself induced the strongest stimulation, TGF-β but not IL-1 still reduced DNA synthesis. bFGF, the most powerful single growth factor used, did not enhance the stimulation of DNA synthesis obtained in the presence of dFCS (Fig 3).

Exogenous Growth Factors and Cell Proliferation in Denuded Vessels

To evaluate the effects of high concentrations of recombinant growth factors on cell proliferation, denuded renal artery segments were exposed for 3 days to 20% dFCS and subsequently for 14 days to CMS-PDS with or without growth factor. Continuous exposure to 20% dFCS was used as a positive control. TGF-β, IL-1, PDGF-AA, PDGF-BB, Ang II, and even bFGF did not stimulate cell proliferation in the periadventitial layer. ET-1, however, resulted in a significant increase in cell numbers. The effect of the peptide was, however, smaller than that of dFCS (Fig 4).

Effects of the Endothelium

In renal and carotid arteries that had not been denuded of endothelium the intramedial DNA synthesis that was stimulated by dFCS during short-term culture was significantly smaller than in segments that had been de-endothelialized (Fig 5).

Conditioned medium collected from intact arterial segments contained significantly more nitrite than medium conditioned by denuded vessels (Fig 6). Nitrite production by intact vessels was not reduced in argi-
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absence of bFGF. BrdU indicates 5-bromo-2'-deoxyuridine.

factor (bFGF; 0.1 to 10 ng/mL) on medial DNA synthesis in
observations in denuded arteries in the absence of TGF-β.
segments were either left intact (+E; D) or mechanically de-
and without endothelium.

Fig 8 illustrates the effects of and potential relation-
the endothelial influence for TGF-β and bFGF, two factors that were shown to modify medial DNA
synthesis. Like the endothelium, exogenous TGF-β
could reduce the strong stimulation of DNA synthesis in
medial cells in 20% dFCS. bFGF, on the other hand,
blunted the inhibitory effect of the endothelium on the
underlying smooth muscle cells.

Discussion

The present findings suggest that responses of
ASMCs to sera and growth factors depend on their
location within the arterial wall, the presence of other
mitogens, and on the endothelium.

In this as in earlier studies,30.32 short-term exposure of
the isolated arterial wall to serum markedly stimulated
medial DNA synthesis, and long-term culture in the
presence of serum resulted in marked cellular prolifera-
tion. The number of medial cells was not altered, but
new cell layers developed at the adventitial edge of the
preparations. Immunocytochemistry previously indicated
that these periadventitial cells display smooth
muscle-like properties such as the presence of vimentin,
desmin, and smooth muscle actins; a continuous
basal membrane containing collagen IV; and deposition
of elastin. Furthermore, a small fraction of them (ap-
proximately 15%) contains smooth muscle α-actin and
smooth muscle myosin heavy chains. This pattern is
somewhat similar to that of neointimal cells. The newly
formed cell layers may be derived from smooth muscle
cells that migrated from the media2-4 or from adventitial
fibroblasts that were modulated into myofibroblasts (for
review, see Reference 38). No immunocytochemical or
ultrastructural criteria are presently available that
clearly distinguish between these possibilities. Based on
the discrepancy between DNA synthesis and cell num-
ber in the media30.32 and on occasional observations of
smooth muscle α-actin-containing cells in holes in the
external elastic lamina, we favor the former possibility.

In that case, the sequence of events during arterial
organ culture, ie, the transient stimulation of medial
DNA synthesis and cell migration and proliferation
outside the media, resembles the response of rat arter-
ies to balloon-catheter injury.3.4

Medial DNA synthesis during short-term culture and
cellular proliferation during long-term culture differed in
their serum dependency. In serum-free medium and in
CMS-PDS a significant medial DNA synthesis was
detected that exceeded the low levels of intra-arterial
DNA synthesis normally observed in intact arteries in
vivo.3.39.40 The molecular origin of this response remains
unclear. However, neither serum-free medium nor
CMS-PDS supported proliferation of periadventitial
cells attracted by a 4-day incubation in the presence of
serum. Furthermore, the effects of exogenous growth
factors differed between the two systems and, from what
is known from cell culture experiments, with dispersed
ASMCs.14,17,21 The most remarkable differences be-
 tween the present organ culture experiments and cell
culture are the lack of stimulatory effect of PDGF on
DNA synthesis and cellular proliferation (except for
PDGF-AA in the presence of IGF-1; see below); the
inability of bFGF to promote extramedial cell prolifera-
tion; and the inhibitory actions of IL-1 on DNA
synthesis. PDGFs are powerful mitogens for isolated
cells.18 In the arterial wall in vivo, however, they act
primarily as chemotactic agents.22 bFGF is also a potent
stimulus for isolated cells.21 In vivo it participates in the
stimulation of medial DNA synthesis after balloon-
catheter injury but not in the proliferation of neointimal
cells.23 Our observation of a stimulatory effect of exog-
 enous bFGF on medial DNA synthesis but not on
proliferation of extramedial cells suggested that not only
temporal differences in the supply of bFGF but also
differences in the responsiveness of medial and extramedial
cells to the growth factor may be responsible for
these phenomena.3.30.32  IL-1 is known to promote prolif-
eration in isolated human saphenous vein cells, especially
when prostanoid synthesis is inhibited.19 We did not ob-
serve a stimulatory action of IL-1 in rat renal arteries
despite blockade of cyclooxygenase. Indeed, the cyto-

![Fig 7. Bar graph showing effects of L-arginine-free medium and Nω-nitro-L-arginine-methyl ester (L-NAME) on nuclear incorporation of 5-bromo-2'-deoxyuridine (BrdU) in isolated arteries of adult Wistar-Kyoto rats. Carotid arteries were either left intact (+E; C) or mechanically denuded (−E; ▲) of endothelium and maintained in culture for 3 days in the presence of 20% dialyzed fetal calf serum (dFCS). Nuclear incorporation of BrdU is shown in the absence of L-arginine (−L-arg), in the presence of L-NAME (L-N-NAME) (200 μmol/L), and under both conditions (−L-arg+L-NAME). Data were expressed as percent labeling and are shown as mean±SEM (n=6). *P<.05 different from preparations with and without endothelium.

![Fig 8. Bar graphs showing effects of transforming growth factor-β (TGF-β; 0.5 to 10 ng/mL) and basic fibroblast growth factor (bFGF; 0.1 to 10 ng/mL) on medial DNA synthesis in isolated arterial wall to serum markedly stimulated medial DNA synthesis, and long-term culture in the presence of serum resulted in marked cellular proliferation. The number of medial cells was not altered, but new cell layers developed at the adventitial edge of the preparations. Immunocytochemistry previously indicated that these periadventitial cells display smooth muscle-like properties such as the presence of vimentin, desmin, and smooth muscle actins; a continuous basal membrane containing collagen IV; and deposition of elastin. Furthermore, a small fraction of them (approximately 15%) contains smooth muscle α-actin and smooth muscle myosin heavy chains. This pattern is somewhat similar to that of neointimal cells. The newly formed cell layers may be derived from smooth muscle cells that migrated from the media2-4 or from adventitial fibroblasts that were modulated into myofibroblasts (for review, see Reference 38). No immunocytochemical or ultrastructural criteria are presently available that clearly distinguish between these possibilities. Based on the discrepancy between DNA synthesis and cell number in the media30.32 and on occasional observations of smooth muscle α-actin-containing cells in holes in the external elastic lamina, we favor the former possibility. In that case, the sequence of events during arterial organ culture, ie, the transient stimulation of medial DNA synthesis and cell migration and proliferation outside the media, resembles the response of rat arteries to balloon-catheter injury.3.4

Medial DNA synthesis during short-term culture and cellular proliferation during long-term culture differed in their serum dependency. In serum-free medium and in CMS-PDS a significant medial DNA synthesis was detected that exceeded the low levels of intra-arterial DNA synthesis normally observed in intact arteries in vivo.3.39.40 The molecular origin of this response remains unclear. However, neither serum-free medium nor CMS-PDS supported proliferation of periadventitial cells attracted by a 4-day incubation in the presence of serum. Furthermore, the effects of exogenous growth factors differed between the two systems and, from what is known from cell culture experiments, with dispersed ASMCs.14,17,21 The most remarkable differences between the present organ culture experiments and cell culture are the lack of stimulatory effect of PDGF on DNA synthesis and cellular proliferation (except for PDGF-AA in the presence of IGF-1; see below); the inability of bFGF to promote extramedial cell proliferation; and the inhibitory actions of IL-1 on DNA synthesis. PDGFs are powerful mitogens for isolated cells.18 In the arterial wall in vivo, however, they act primarily as chemotactic agents.22 bFGF is also a potent stimulus for isolated cells.21 In vivo it participates in the stimulation of medial DNA synthesis after balloon-catheter injury but not in the proliferation of neointimal cells.23 Our observation of a stimulatory effect of exogenous bFGF on medial DNA synthesis but not on proliferation of extramedial cells suggested that not only temporal differences in the supply of bFGF but also differences in the responsiveness of medial and extramedial cells to the growth factor may be responsible for these phenomena.3.30.32 IL-1 is known to promote proliferation in isolated human saphenous vein cells, especially when prostaglandin synthesis is inhibited.19 We did not observe a stimulatory action of IL-1 in rat renal arteries despite blockade of cyclooxygenase. Indeed, the cyto-
kine reduced medial DNA synthesis in CMS-PDS and PDS. Not only interspecies and regional differences must be taken into account to explain these discrepancies but also differences in susceptibility to mediators between medial cells and extramedial cells.

During the past few years it has become increasingly clear that several exogenously supplied mitogens do not act directly but rather through stimulation of the production of autocrine growth factors or their receptors. This has been documented repeatedly for PDGF and PDGF receptors. Consequently, the inability of the arterial wall to respond to various growth factors may find its origin in the lack of responsiveness to this family of autocrine agents. Complex processes such as DNA synthesis and proliferation may, on the other hand, require the coordinated action of several factors. In this respect mitogens have been classified as competence and progression factors. IGF-1 belongs to this latter class. In the present study this factor unmasked significant stimulation of DNA synthesis by PDGF-AA and blunted the inhibitory action of IL-1. Future studies should thus concentrate on combinations of mitogens rather than on single factors. Furthermore, the effects of mitogens on DNA synthesis and cellular proliferation may be obscured by enhanced synthesis of inhibitory agents such as prostaglandins and TGF-β.

The most powerful modulator of medial DNA synthesis observed in the present study was the endothelium. Cell culture experiments indicated that the endothelium can secrete all the factors that we tested during arterial organ culture. Of these, IL-1 and TGF-β inhibited DNA synthesis while bFGF and ET-1 stimulated the response. Thus, with respect to its effects on media contractility, the endothelium has at least the potential to both inhibit and stimulate growth responses in underlying smooth muscle. This conclusion was also reached when effects of endothelium removal in various arterial organ culture experiments were compared.

In the present study the effects of IL-1 were used to verify the nature of the endothelial inhibition of medial DNA synthesis. Under certain conditions the cytokine can stimulate expression of NO synthase in several cell types, including smooth muscle cells. NO synthase is normally constitutively expressed in endothelial cells, and its reaction product, NO, accounts for the majority of the effects of endothelium on arterial contractility. The similarity of effects of IL-1 and endothelium on medial DNA synthesis along with the observed production of nitrite, a stable degradation product of NO, are in line with a possible role of the radical in endothelial modulation. However, differences between vessels with and without endothelium persisted in arginine-free culture medium and were not abolished by an arginine analogue that inhibits NO synthase. In earlier experiments we observed that methylene blue, a scavenger of NO and an inhibitor of NO action on arterial contractility, does not stimulate DNA synthesis in rat arteries with endothelium. In addition, while IL-1 displayed an inhibitory effect during moderate stimulation of DNA synthesis (ie, in the presence of CMS-PDS or PDS), the cytokine was ineffective in the presence of a high concentration of FCS. The endothelium, however, also markedly reduced the response to this strong stimulus. Combined, the findings indicate that phenomena other than endothelium-derived NO and IL-1 participate in endothelial inhibition of medial DNA synthesis. A role for TGF-β may be worth considering. In the present experiments, TGF-β reduced DNA synthesis under various conditions. Furthermore, TGF-β and bFGF counteract one another in several systems, and we found that bFGF can potently inhibit the endothelial effect on medial DNA synthesis. Clearly, additional experiments are required to firmly establish the role of TGF-β.

In summary, factors that may be produced by smooth muscle and endothelial cells were found to affect DNA synthesis and cellular proliferation in isolated arteries. Both stimuli and inhibitors were identified. They affected medial DNA synthesis and extramedial cell proliferation differently. It was also confirmed that the endothelium can markedly suppress stimulated DNA synthesis in rat arteries. It is unlikely that this is primarily due to endothelium-derived prostaglandins, NO, or IL-1.

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