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

Paul M.H. Schiffers, Gregorio E. Fazzi, Dorette van Ingen Schenau, Jo G.R. De Mey

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 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 more marked 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 endothelin-derived prostanoids, 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. These approaches indicate that vascular cells can produce peptide-like growth factors, cytokines, and vasoactive agents that can interfere with growth responses. This is not restricted to ASMCs but applies to endothelial cells as well. 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. Many agents have been shown to promote proliferation of isolated ASMCs in vitro, but this does not necessarily predict their activity in the vascular wall. 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 components and the multifac-}

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From the Vascular Biology Laboratory, Department of Pharmacology, and Cardiovascular Research Institute Maastricht, University of Limburg, Maastricht, the Netherlands.

Correspondence to Jo G.R. De Mey, Department of Pharmacology, University of Limburg, PO Box 616, 6200 MD Maastricht, the Netherlands.
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 demonstrated⁴⁰ 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 serotonin⁴¹ and angiotensins (P.M.H. Schiffer, May 1993, unpublished observations). For this reason, the serum was dialyzed (cutoff point, 6 kDa) against three changes of phosphate buffer, pH 7.4, at 4°C for 24 hours.

Both plasma-derived serum (PDS) and CM Sephadex-PDS (CMS-PDS) were prepared by a method described by Vogel et al.⁴² 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; K₂HPO₄ 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 (50×2 cm), and approximately eight times the original volume. This CMS-PDS was dialyzed against three changes of 0.1 mol/L Tris-HCl, pH 7.4, as eluants. The eluate was concentrated back to the original volume. This CMS-PDS and PDS were heated to 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 N²-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.⁴² All segments were mounted on a 0.25-mm sterile wire (Dentaurum) 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 glutamine (GIBCO), 100 IU/mL penicillin (Gist-Brocades), 100 μg/mL streptomycin (Pharmachem), and either 20% dialyzed FCS (dFCS; Seralab), 31% rat PDS, or 5% human serum (HSA) (Sera) at a final concentration of 20 mmol/L L-arginine content, 250 μmol/L glutamine (GIBCO), 100 IU/mL penicillin, 10 ng/mL TGF-β, or 10 ng/mL bFGF. The concentrations of these mediators have been reported to be maximal with respect to stimulation of growth in vitro. (KRB: composition in mmol/L, NaCl 118.5, KCl 4.7; MgSO₄ 1.2; K₂HPO₄ 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-HCl, pH 7.4, at 4°C for 24 hours. Part of this PDS was then applied to a CM Sephadex column (50×2 cm), and approximately eight times the original volume. This CMS-PDS was dialyzed against three changes of KRB for 24 hours. Both CMS-PDS and PDS were heated to 56°C for 30 minutes, centrifuged at 3000 rpm at 4°C for 20 minutes, filtered, and stored at −70°C until use.

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Nuclear incorporation of BrdU was evaluated by immunohistochemistry of cross sections.³¹-³⁵ To quantify the extent of DNA synthesis, the percentage of nuclear profiles that stained for BrdU was determined in the tunica media.

In the present study, we confirmed that medial DNA synthesis was significantly higher in arterial segments denuded of endothelium than in intact arteries.²⁹ To evaluate whether exogenous nitric oxide (NO), DNA synthesis was measured in carotid arteries that were either left intact or denuded of endothelium and that had been exposed to 20% dFCS for 3 days of culture in the absence or presence of L-arginine (250 μmol/L) or L-NAME (200 μmol/L). The production of nitrate (a stable degradation product of NO) was also measured in these experiments in the conditioned media.²⁶ In brief, 500-μL samples were mixed with an equal volume of Griess reagent (0.5% sulfanilamide/0.05% N₁-naphthyl[ethylendiamine dihydrochloride) and incubated at room temperature for 10 minutes. The optical densities were measured at 550 nm, and NO₃⁻ concentrations were determined by using NaNO₂ as a standard (1 to 100 μmol/L) and water as a blank. Background NO₃⁻ values of media were determined and subtracted from the experimental values.

Statistics

Data are shown as mean±SEM. Statistical significance of differences was evaluated by a one- or two-way ANOVA where applicable. For multiple preplanned comparisons, the ANOVA was followed by Bonferroni’s test.³⁷ A probability of <.05 was taken to denote statistical significance.

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...
BrdU Labeling (%)  
0 10 20 30 40 50  
  
**Fig 1.** Bar graph showing effects of different sera on medial DNA synthesis in isolated and denuded renal (RA; •) and carotid (CA; •) arteries during 3 days of organ culture. The percentage of 5-bromo-2'-deoxyuridine (BrdU) labeling is shown for 20% 
dialyzed fetal calf serum (dFCS), 5% plasma-derived serum (PDS), 5% CM Sephadex (CMS)-PDS, and serum-free medium (O). Data are expressed as mean±SEM (n=6). *P<.05 between 
renal and carotid arteries.

(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 me-
dium). In denuded carotid artery segments, BrdU label-
ing of the media was significant after 3 days of incubation 
in serum-free medium (1.9±0.6%/3 d) and was stimu-
lated 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 
asence 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 continu-
ous 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 were evaluated during mild 
(CMS-PDS), moderate (PDS), and strong (dFCS) stim-
ulation, and these agents were also tested in combina-
tion 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 en-
hanced DNA synthesis in CMS-PDS. It did not affect 
the inhibitory action of TGF-β but abolished the inhibi-
tory 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 syn-
thesis (Fig 3).
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 arginine-free culture medium but was decreased by 200 μmol/L L-NAME (an inhibitor of NO synthase) (Fig 6). Furthermore, denuded vessels that were exposed to IL-1 produced more nitrite than denuded vessels maintained in the absence of the cytokine. Nitrite production stimulated by IL-1 required the presence of exogenous l-arginine (Fig 6). These findings suggest basal activity of endothelial NO synthase in intact vessels and induction of NO synthase outside the endothelium by IL-1. Because IL-1 can reduce medial DNA synthesis (Fig 3), it is possible that endothelial effects on intra-arterial DNA synthesis may be mediated by NO. However, differences between vessels with and without endothelium with respect to BrdU labeling persisted in the absence of exogenous l-arginine and were not affected by L-NAME (Fig 7).

Effects of 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, noted in the presence of PDS was reduced by TGF-β (Fig 4). Differential stimulation of DNA synthesis obtained in the presence of dFCS (Fig 3).

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

Exogenous growth factor (bFGF; 10 ng/mL). Dialyzed fetal calf serum was used as a positive control. Data are shown as mean±SEM (n=6). *P<.05 different from observations in the absence of mediator.

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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 media 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 media 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, i.e., 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.4,5

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.3-5 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 the phenocopies of 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-

**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 renal arteries from adult Wistar-Kyoto rats. Arterial segments were either left intact (+E; □) 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 in isolated arteries of adult Wistar-Kyoto rats. Carotid arteries were either left intact (+E; D) or mechanically de-
kinine 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.\(^{41,42}\) 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.\(^{43}\) 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-\(\beta\).\(^{19,25,26}\) The former do not interfere with the present results because all experiments were performed in the presence of the cyclooxygenase inhibitor indomethacin. Other growth inhibitors, however, cannot be excluded.

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.\(^{10-15}\) Of these, IL-1 and TGF-\(\beta\)-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.\(^{44}\)

In the present study the effects of IL-1 were used to verify the nature of the endothelial inhibition of medial DNA synthesis. A synthetic cytokine can stimulate expression of NO synthase in several cell types, including smooth muscle cells.\(^{15}\) NO synthase is normally constitutively expressed in endothelial cells,\(^{15}\) and its reaction product, NO, accounts for the majority of the effects of endothelium on arterial contractility.\(^{45}\) 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-\(\beta\) may be worth considering. In the present experiments, TGF-\(\beta\) reduced DNA synthesis under various conditions. Furthermore, TGF-\(\beta\) and bFGF counteract one another in several systems,\(^{12,17,25,43}\) 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-\(\beta\).

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 stimulii 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.

References

or two effects with one unknown cause? Circulation. 1991;84(suppl VI):VI-2-VI-16.


27. Southgate K, Newby AC. Serum-induced proliferation of rabbit aortic smooth muscle cells from the contractile state is inhibited by 8-Br-cAMP but not 8-Br-cGMP. Atherosclerosis. 1990;82:113-123.


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