Agonist-mediated Tissue Factor Expression in Cultured Vascular Smooth Muscle Cells

Role of Ca^{2+} Mobilization and Protein Kinase C Activation

Mark B. Taubman,*‡ Jonathan D. Marmur,‡ Claire-Lise Rosenfield,* Arabinda Guha,‡ Steven Nichtberger,‡ and Yale Nemerson‡§

*Molecular Medicine Unit, *Brookdale Center for Molecular Biology and ‡Department of Medicine; and §Department of Biochemistry, Mount Sinai School of Medicine, New York 10029*

Abstract

Tissue factor (TF) is a low molecular weight glycoprotein that initiates the clotting cascade and is considered to be a major regulator of coagulation, hemostasis, and thrombosis. TF is not expressed in the intima or media of normal adult blood vessels. Accordingly, it has been hypothesized that the initiation of intravascular coagulation may require the "induced" expression of TF in the vessel wall. We report that TF mRNA and protein are rapidly and markedly induced in early and late passaged vascular smooth muscle cells (VSMC) by growth factors (serum, platelet-derived growth factor, epidermal growth factor), vasoactive agonists (angiotensin II), and a clotting factor (α-thrombin). The induction of TF mRNA by these agents is dependent upon mobilization of intracellular Ca^{2+} and is blocked by Ca^{2+} chelation. In contrast to other growth factor–responsive genes, such as *KC* and c-*fos*, downregulation of protein kinase C activity by prolonged treatment with phorbol esters fails to block agonist-mediated TF induction. This raises the possibility that protein kinase C activation may not be necessary for TF mRNA induction in VSMC. VSMC may play a role in the generation or propagation of thrombus through the induction of TF, particularly in settings, such as those associated with acute vessel injury, where the endothelium is denuded and the VSMC are exposed to circulating blood. (*J. Clin. Invest.* 1993. 91:547–552.) Key words: vascular smooth muscle • tissue factor • growth factors • thrombosis • gene expression

Introduction

Tissue factor (TF)[1] is a low molecular weight glycoprotein that initiates the clotting cascade and is considered to be a major regulator of coagulation, hemostasis, and thrombosis (1, 2). Unlike other coagulation factors, TF is not normally found in the circulation, but is located on the plasma membranes of cells not usually in contact with the blood. Human TF consists of three domains: a short cytoplasmic domain of 21 residues, a single transmembrane domain of 23 residues, and a large extracellular domain of 219 residues. TF binds to Factor VII/VIIa, and the resulting complex acts as a catalyst for the conversion of Factors IX and X to IXa and Xa, respectively, thus triggering the clotting cascade.

TF is not expressed in the intima or media of normal adult blood vessels (3, 4). This has led some to hypothesize that the initiation of intravascular coagulation requires the "induced" expression of TF (3). Wilcox et al. (4) examined normal internal mammary arteries using both immunohistochemistry with an anti–human TF antibody and in situ hybridization with a human TF RNA probe. Endothelial cells did not contain detectable TF mRNA and protein in any sample examined. The media from all arteries tested (including coronary and aorta) contained only rare cells with TF mRNA and none with TF protein. In contrast, the adventitial fibroblasts showed intense TF immunostaining and mRNA hybridization in all arterial samples. When atherosclerotic plaques from carotid endarterectomy specimens were examined by similar methods, TF mRNA and protein remained absent from the endothelium, but were identified in mesenchymal-like intimal cells (presumably vascular smooth muscle cells [VSMC]) as well as in foam cells and monocytes adjacent to cholesterol clefts, and in the extracellular matrix. These authors suggested that the induction of TF in the atherosclerotic plaque might contribute to the hyperthrombotic state of atherosclerotic vessels. In addition to the above work, functional TF has also been demonstrated in ballooned (deendothelialized) human and rabbit arteries, suggesting that its induction may also contribute to the thrombotic response accompanying acute vessel injury (5).

Experiments in cell culture have demonstrated TF to be highly inducible by a variety of agents. In endothelial cell culture, TF mRNA and/or procoagulant activity is induced by phorbol esters (6–8), tumor necrosis factor (6, 9, 10), endotoxin (7, 11), interleukin 1 (12, 13), and α-thrombin (14). Similar results have been found in monocytes, where TF mRNA and/or procoagulant activity is stimulated by a variety of mediators of inflammation and antigen-specific cellular immune responses (for review, see reference 2). In mouse fibroblasts, TF has been found to be a member of the class of "immediate early" genes induced by serum and growth factors, including platelet-derived growth factor (PDGF), fibroblast growth factor, and transforming growth factor β (15, 16). These authors have speculated that as an immediate early gene, TF might have a growth-related function in addition to its role in coagulation. Although Maynard et al. (17) described the

Address correspondence to Mark B. Taubman, M.D., Box 1126, Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, NY 10029.

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1. *Abbreviations used in this paper:* $[Ca^{2+}]_i$, intracellular free calcium concentration; CS, calf serum; EGF, epidermal growth factor; PDBu, phorbol 12,13-dibutyrate; PDGF, platelet-derived growth factor; TF, tissue factor; VSMC, vascular smooth muscle cells.

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presence of TF coagulant activity in cultured VSMC, the regulation of TF in VSMC has not previously been explored.

We now report that TF mRNA and protein are rapidly and markedly induced in cultured VSMC by growth factors, vasoactive agonists, and clotting factors. Induction by each of these agonists is dependent upon mobilization of intracellular Ca^{2+} and can be blocked by Ca^{2+} chelation. In contrast, induction of TF mRNA by these agents may be independent of protein kinase C activation. The induction of TF in cultured VSMC suggests that these cells may play a role in the generation or propagation of thrombus.

Methods

Growth factors and other reagents. PDGF (50% pure) and mouse epidermal growth factor (EGF, receptor grade) were purchased from Collaborative Research Inc. (Bedford, MA). Angiotensin II, bovine insulin, phorbol 12,13-dibutyrate, and phorbol 12-myristate 13-acetate were obtained from Sigma Chemical Co. (St. Louis, MO). Ionomycin, Quin 2/AM, and BAPTA/AM were obtained from Calbiochem Corp. (San Diego, CA). Human α-thrombin was a generous gift of Dr. John W. Fenton II (Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany, NY).

Cell culture. VSMC were isolated from the thoracic aortas of 200–300-g male Sprague-Dawley rats by enzymatic dissociation (18). Cells were grown in DME supplemented with 10% heat-inactivated calf serum (CS), 100 U/ml penicillin and 100 μg/ml streptomycin, and serially passaged before reaching confluence. To produce quiescence, cells were incubated in DME with 0.3% CS for 48–72 h. Under these conditions, incorporation of [^{3}H]thymidine into DNA was < 15% of that seen with 10% serum. VSMC expressed smooth muscle α-actin mRNA (as determined by Northern blot analysis using a probe encoding the 3′ untranslated end of rat smooth muscle α-actin) and protein (as determined by two-dimensional gel electrophoresis and immunostaining with antibody against α-actin) as well as mRNA encoding smooth muscle α-tropomyosin in all passages used. These cells also maintained high levels of functioning receptors for Ang II (19) and α-thrombin (20) even at later (> 20) passages. All experiments depicted in the figures were performed at least three times, using VSMC from different subculture passages. Unless otherwise indicated, experiments shown were derived from passages 5–10.

To measure TF induction in the absence of a change in intracellular Ca^{2+} ($[Ca^{2+}]_i$), quiescent VSMC were washed twice in Tris-buffered saline solution buffer (130 mM NaCl, 5 mM KCl, 1.0 mM $MgCl_2$, 0.25 mg/ml BSA, 10 mM glucose, and 20 mM Hepes, pH 7.4) and then equilibrated for 30 min at 37°C in room air in the same buffer containing 4 mM EGTA and either 10 μM Quin 2/AM or BAPTA/AM. Agonists were then added directly to some cultures and incubated at 37°C in room air for the times indicated. Under these conditions, agonist-mediated Ca^{2+} mobilization is completely blocked (19, 21). In some experiments, incubations were performed in 4 mM EGTA without Quin 2/AM.

RNA preparation and blot hybridization. Total RNA was extracted from VSMC by the guanidinium isothiocyanate/CsCl procedure (22). Agarose gel electrophoresis, transfer to nitrocellulose and hybridization to ^{32}P-labeled DNA were as previously described (23). Prehybridization and hybridization were performed at 42°C. Final washes for all blots were in 0.1 × standard saline citrate (SSC) (1 × = 0.15 M NaCl/0.015 M sodium citrate, pH 7) and 0.1% SDS at 65°C for 1 h. The full-length insert from mouse TF (15) was labeled by random oligomer priming to a specific activity of > 10^8 cpm/μg and used at 2 × 10^6 cpm/ml. As a control, filters were also hybridized with cDNA encoding the constitutively expressed smooth muscle myosin regulatory light chain (24). All experiments shown in the figures represent RNA blots in which all lanes had equal loading as confirmed both by staining of 18s and 28s ribosomal RNA with ethidium bromide and by hybridization with the regulatory light chain.

Analysis of tissue factor activity. Quiescent and stimulated VSMC cultures were washed twice with 20 mM Hepes, pH 7.6, 140 mM NaCl, and 4 mM EDTA, scraped into 1 ml of 20 mM Hepes, pH 7.6, 140 mM NaCl (4°C), and sonicated at 20 kHz for 20 s (model W375; Heat Systems-Ultrasonics Inc., Farmingdale, NY). 50-μl aliquots were assayed in duplicate for TF activity using modifications of the standard two-stage procedure (25, 26). Each aliquot was incubated at 37°C for 1 min with 50 μl of rat Factor VII-X concentrate (see below) and 50 μl of a 5 mM $CaCl_2$, 12.5 μM phosphatidyl serine/phosphatidyl choline (30/70; wt/wt) solution. 50 μl of human plasma (derived from an individual donor, aliquoted into single-use samples, and stored at −80°C) was then added at 37°C and the clotting time was recorded. To generate a standard TF activity curve, a mouse brain homogenate was prepared as follows: 1.5 g of stripped mouse brain (Pel-Freez Biologicals, Rogers, AR) was suspended in 15 ml isotonic saline, disrupted by sonication, centrifuged at 1,000 g × 15 min, and the supernatant aliquoted and stored at −80°C. The protein concentration of the homogenate was determined using Bradford reagent (protein assay; Bio-Rad Laboratories, Richmond, CA) with BSA as a standard. When assayed as above, 1–30 μg of homogenate generated a linear curve when plotted on a semi-log scale. The amount of protein required to produce a clotting time of 25 s was arbitrarily assigned a value of 100 activity units. TF activity in VSMC samples was expressed as activity units per mg of protein.

A crude concentrate of Factors X, VII, and VII_a was prepared from mouse serum (Pel-Freez Biologicals) as previously described (26) with minor modifications. 7.6% sodium citrate (1 ml) and 1 M $BaCl_2$ (2 ml) were added to 20 ml of serum, stirred for 30 min at 4°C, and precipitated by centrifugation at 3,000 g × 15 min. The precipitate was washed × 2 with 5 ml of 5 mM $BaCl_2$, resuspended in 2 ml of 25% $(NH_4)_2SO_4$, 50 mM Tris-HCl (pH 7.5), and 0.1 M NaCl, and stirred for 30 min at 4°C. After centrifugation at 3,000 g × 15 min, the supernatant was collected, dialyzed overnight at 4°C against Tris-buffered saline. This stock concentrate was aliquoted and stored at −80°C. For TF assays, the stock was diluted 1:10.

Results

Induction of TF mRNA in VSMC culture. To investigate the induction of TF mRNA in cultured VSMC, quiescent cells were treated with PDGF (50% pure; Collaborative Research Inc.) for the times indicated. Total RNA was harvested and RNA blot hybridization was performed using the full-length mouse TF cDNA (15). mRNA corresponding to an ≈ 2-kb TF species was either not detectable (as depicted in Fig. 1 *A*) or barely detectable in quiescent VSMC. PDGF induced a rapid and marked rise in TF mRNA levels, beginning at ≈ 15 min and peaking between 75 and 90 min. The induction of TF mRNA was particularly short-lived in VSMC, with levels returning to baseline within 3–4 h. Quiescence was not a requisite for TF induction. VSMC cultures undergoing log-phase growth (75% confluence) had little (as shown in Fig. 1B) or no detectable TF mRNA 48 h after feeding with standard growth medium (DME + 10% CS). Upon addition of fresh DME + 10% CS, there was a rapid induction of TF mRNA, with time course and levels similar to that found in quiescent VSMC following PDGF or serum stimulation. Neither the degree of confluence (50–100%) nor the passage number (1–15) had any significant effect on the level or time course of TF mRNA induction by serum or PDGF (data not shown). Identical results were obtained using VSMC derived from 3 different aortic preparations.

A variety of agents appear capable of inducing TF mRNA in VSMC. As shown in Fig. 2, marked and short-lived induc-

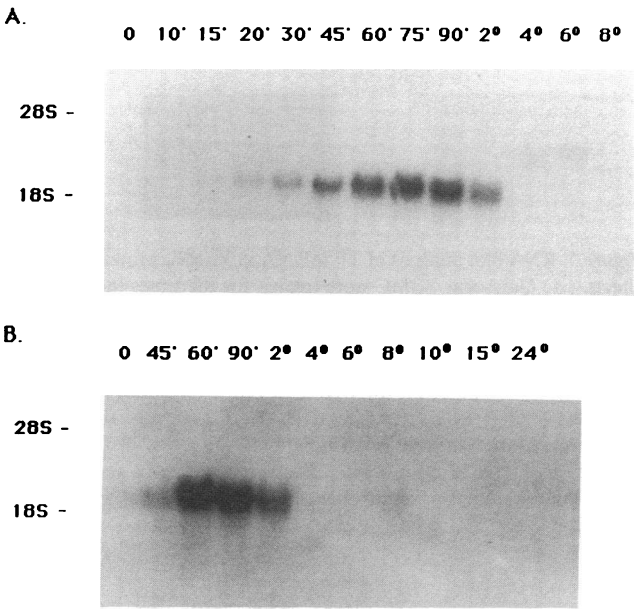

Figure 1. RNA blot analysis of TF mRNA in rat aortic VSMC: time course of induction. (*A*) Quiescent VSMC (incubated in DME with 0.3% CS for 48 h; 0) were treated for the times indicated with PDGF (5 half-maximal U/ml 50% pure PDGF, Collaborative Research Inc). (*B*) VSMC in log-phase growth (75% confluency) were treated with PDGF for the times indicated beginning 48 h after the last change of medium (DME with 10% CS; 0). Blots, containing 10 μg of total RNA/lane, were hybridized to the full-length mouse TF cDNA and washed at 0.1 × SSC, 65°C. The hybridizing band corresponds to the 2-kb TF species.

tion of TF mRNA was seen with 10% CS, angiotensin II (100 nM), α-thrombin (25 nM), and EGF (200 ng/ml), all of which have growth-promoting properties for cultured rat aortic VSMC.

Induction of TF protein in VSMC culture. To establish that the rise in TF mRNA in VSMC was accompanied by the synthesis of TF protein, TF activity was measured under similar conditions to that associated with TF mRNA induction. As shown in Fig. 3, minimal TF activity was measured in untreated, quiescent VSMC. In contrast, after induction with 10% CS or angiotensin II, there was a marked increase in TF activity, beginning at ≈ 1 h, peaking at 2–8 h, and then slowly returning to baseline.

Signal transduction and TF induction in VSMC. Angiotensin II, α-thrombin, and PDGF cause a concentration-dependent increase in $[Ca^{2+}]_i$ in VSMC due to mobilization of intracellular Ca^{2+} stores (20, 21, 27). Treatment of VSMC with the combination of the intracellular and extracellular Ca^{2+} chelators, Quin 2/AM and EGTA, completely inhibits the change in $[Ca^{2+}]_i$ in VSMC. In addition, this protocol has been shown to block the induction of c-*fos* and *KC* mRNA by angiotensin II and α-thrombin (19, 21). As shown in Fig. 4 *A*, extracellular Ca^{2+} chelation with EGTA had a small inhibitory effect on the induction of TF mRNA by PDGF. However, the combination of BAPTA/AM (or Quin 2/AM) and EGTA completely inhibited the induction of TF mRNA by PDGF. The inhibition of TF mRNA induction by the combination of intracellular and extracellular Ca^{2+} chelation is not a generalized phenomenon of all early growth factor–induced genes, in that the same conditions failed to inhibit the induction of *JE* mRNA by PDGF (23). As shown in Fig. 4 *B*, treatment with Quin2/AM and EGTA also inhibited the induction of TF mRNA by α-thrombin, angiotensin II, and the phorbol ester, PMA. These results suggest that Ca^{2+} mobilization may represent a common pathway by which a wide range of agents activate TF gene expression in VSMC. As predicted by the results of the Ca^{2+} blockade experiments, the Ca^{2+} ionophore, ionomycin (1 μM), induced TF mRNA with a time course similar to that seen for other agonists (Fig. 4 *C*).

Activation of protein kinase C plays an important role in the induction of a number of growth-related genes, such as

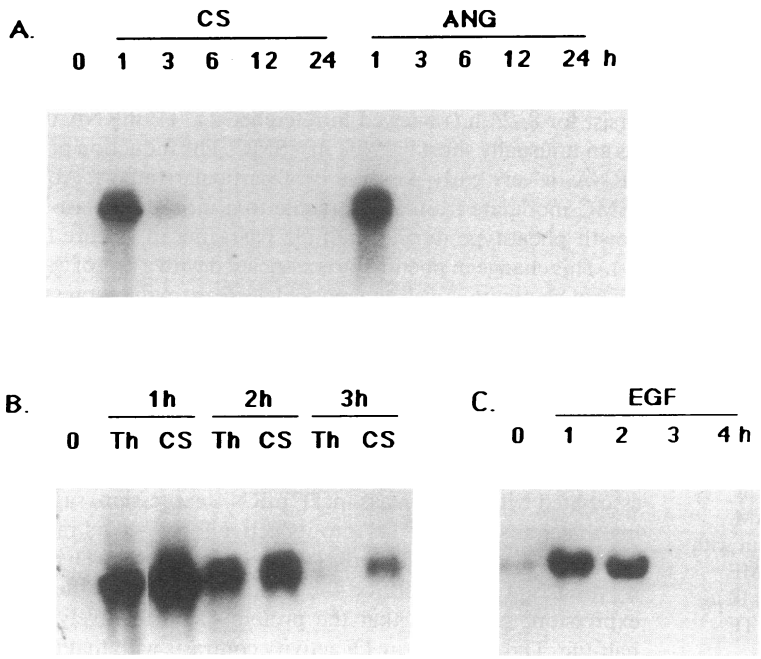

Figure 2. RNA blot analysis of TF mRNA in cultured VSMC: agonist effects. Figure represents a composite of three different experiments. VSMC (made quiescent by incubation in DME + 0.3% CS for 48 h; 0) were treated with the following agonists for the times indicated: (*A*) 10% CS (*CS*) or 10^{-7} M angiotensin II (*ANG*); (*B*) 25 nM α-thrombin (*Th*) or 10% CS (*CS*); or (*D*) 200 ng/ml epidermal growth factor (*EGF*). Blots, containing 10 μg of total RNA/lane, were hybridized to the full-length mouse TF cDNA and washed at 0.1 × SSC, 65°C.

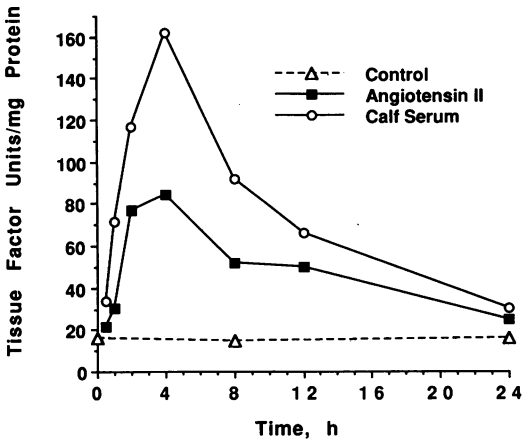

Figure 3. TF activity in cultured VSMC. Quiescent VSMC (time 0), treated for the times indicated with either 10% CS or 10^{-6} M angiotensin II. Each point represents the average of four measurements and are expressed as total activity per mg of protein. Units correspond to the amount of Factor Xa activity generated by VSMC as described in Methods.

c-*fos* and c-*myc* (28, 29). PDGF, angiotensin II, and α-thrombin all stimulate protein kinase C (19, 20, 27). Phorbol 12-myristate 13-acetate (PMA; 250 ng/ml), a potent activator of protein kinase C, induced TF mRNA with a time course and levels similar to those induced by other agonists (Fig. 5 *A*). To test whether protein kinase C played a role in the induction of TF mRNA by growth factors and vasoactive agonists, VSMC were pretreated for 24 h with phorbol 12,13-dibutyrate (PDBu). As previously reported (19), this pretreatment decreases cytosolic protein kinase C activity by 80–90% when measured by phosphorylation of histone III-S. In addition, it abolishes PMA- and angiotensin II–stimulated phosphorylation of the 76,000-D protein kinase C substrate. Pretreatment of VSMC with PDBu completely abolished the rise in TF mRNA levels in response to PMA (Fig. 5 *B*), demonstrating that this treatment was effective in blocking the induction of TF by a specific activator of protein kinase C. In contrast, PDBu pretreatment had no significant effect on the induction of TF mRNA by 10% serum, angiotensin II, α-thrombin, EGF, or ionomycin. As discussed further below, these results suggest that activation of protein kinase C may not be important in the induction of TF mRNA by these agents.

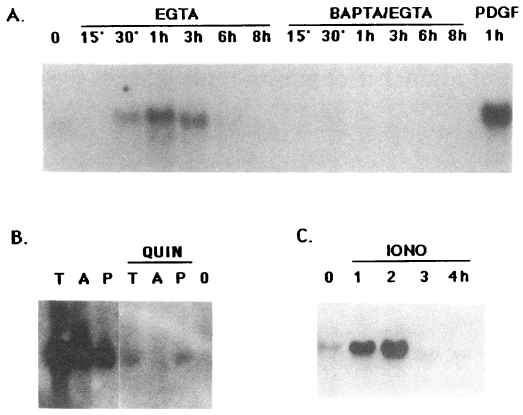

Figure 4. RNA blot analysis of TF mRNA in VSMC: calcium effects. (*A*) Quiescent VSMC were left untreated (0) or exposed for 1 h to PDGF (5 half-maximal U/ml of 50% pure PDGF). Duplicate cultures were treated with PDGF for the times indicated in Ca^{2+}-free buffer (see Methods) containing either 4 mM EGTA alone (*EGTA*) or 4 mM EGTA + 10 μM BAPTA/AM (*BAPTA/EGTA*). (*B*) Quiescent VSMC (*O*) were treated for 1 h with 25 nM α-thrombin (*T*), 10^{-7} M angiotensin II (*A*), or 250 nM PMA (*P*) in DME or in Ca^{2+}-free buffer containing 4 mM EGTA + 10 μM Quin2/AM (*QUIN*). (*C*) Quiescent VSMC cultures were treated for 5 min with 1 μM ionomycin (*IONO*), washed, and then incubated in DME (without ionomycin) for the times indicated. Blots, containing 10 μg of total RNA/lane, were hybridized to the full-length mouse TF cDNA and washed at 0.1 × SSC, 65°C.

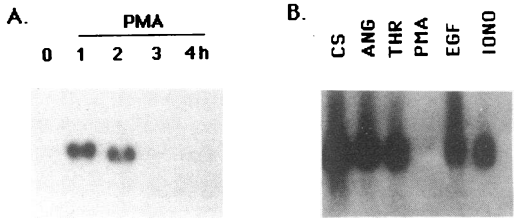

Figure 5. RNA blot analysis of TF mRNA in VSMC: protein kinase C effects. (*A*) Quiescent VSMC were treated for the times indicated with the phorbol ester, PMA. (*B*) Quiescent VSMC were pretreated for 24 h with 1 μM phorbol 12,13 dibutyrate in DME + 0.3% CS to downregulate protein kinase C, and then exposed for 1 h to 10% CS (*CS*), 10^{-7} M angiotensin II (*ANG*), 25 nM α-thrombin (*THR*), 250 nM PMA (*PMA*), 200 ng/ml EGF (*EGF*), or 1 μM ionomycin (5 min exposure followed by washing and incubation in DME without ionomycin for 50 min; *IONO*). Blots, containing 10 μg of total RNA/lane, were hybridized to the full-length mouse TF cDNA and washed at 0.1 × SSC, 65°C.

Discussion

The data described above demonstrate that TF gene expression is highly regulated in cultured VSMC. The time course of growth factor–induced TF mRNA expression is similar to that reported for 3T3 fibroblasts (15), but considerably shorter than that found in most other studies employing endothelial cells, human fibroblasts or monocytes, where elevated levels persist for 8–24 h (reviewed in reference 2). TF mRNA thus has an unusually short half-life in VSMC. The induction of TF mRNA in very early passages is of particular import. Aortic VSMC modulate from a contractile to a more fibroblast-like growth phenotype during multiple passaging in culture (30, 31). This change in phenotype is associated with a loss of actin-myosin filaments as well as a marked decrease in the expression of smooth muscle α-actin mRNA (32). The finding that TF mRNA is induced in very early passages (passages 1–5) strongly argues that this induction is not an artifact of cell culture.

The induction of TF mRNA is associated with a marked increase in TF activity. The rise in TF activity is temporally associated with the increase in TF mRNA expression, suggesting that translation of TF into its active form occurs rapidly following elevation of TF mRNA levels. In contrast, TF activity persists at high levels for 4–8 h after the fall in TF mRNA expression, suggesting that the protein has a relatively long half-life. The early rise in TF activity contrasts with that previ-

ously reported for smooth muscle cells derived from human saphenous veins, where there was a 24-h delay in TF activity after serum stimulation (17). The early induction of TF mRNA and activity in VSMC derived from the arterial tree suggests that arterial VSMC may be more specialized than venous VSMC in their ability to mediate acute thrombosis. Alternatively, the difference in the time course of TF induction may reflect a species-specific phenomenon (i.e., human vs. rat).

An immediate response to a variety of agonists, including PDGF, angiotensin II, and α-thrombin, is the activation of phospholipase C. This enzyme hydrolyzes phosphatidylinositol-4,5-bisphosphate to generate the "second messengers," diacylglycerol and inositol trisphosphate. Diacylglycerol stimulates protein kinase C, whereas inositol trisphosphate mobilizes Ca^{2+} from intracellular stores. Activation of protein kinase C and mobilization of intracellular Ca^{2+} have been shown to be involved in the induction of a number of early growth factor–inducible genes (19, 20, 27–29, 33). The Ca^{2+} ionophore, ionomycin, markedly induced TF mRNA in cultured VSMC. More significantly, the induction of TF in VSMC by a wide range of agonists, including PDGF, angiotensin II, and α-thrombin, was dependent upon mobilization of intracellular Ca^{2+}. It has recently been shown, in bovine fibroblasts, pericytes, and kidney cells, that Ca^{2+} ionophore markedly increases TF activity on the cell membrane. This increase in activity is due to a process of deencryption, whereby increased intracellular Ca^{2+} causes a change in TF accessibility on the cell membrane, exposing previously encrypted (e.g., inactive) TF molecules (34). Thus, increases in intracellular Ca^{2+} may play a dual role in TF regulation, modulating both TF gene expression and TF protein activity.

The mechanism by which intracellular Ca^{2+} mobilization modulates the activation of TF remains to be determined. Ca^{2+} may play a role in TF gene induction by directly activating a Ca^{2+}-response element or by modulating the activity of a *trans*-acting factor(s), such as one that binds to a serum- or PDGF-response element. Ca^{2+} may also be a necessary co-factor in one or more steps of the signaling pathway leading to TF gene expression or regulation of TF mRNA stability. For example, it has recently been reported that the inhibition of calmodulin by myosin light chain kinase peptide attenuated the expression of TF activity in human umbilical vein endothelial cells (35). This report, along with several earlier reports employing very high doses of calmodulin antagonists (36, 37), suggests that the effect of Ca^{2+} on TF induction may involve a calmodulin-sensitive pathway.

It has previously been shown that phorbol esters, which are activators of protein kinase C, induce high levels of TF mRNA in human endothelial cells and peripheral leukocytes (6–8). This report demonstrates that phorbol esters also induce TF mRNA in cultured VSMC, although the duration of TF mRNA expression is considerably shorter than that reported for endothelial cells or leukocytes. PDBu-mediated downregulation of cytosolic protein kinase C activity by 80–90% had no effect on the induction of TF mRNA by angiotensin II, α-thrombin, PDGF, or Ca^{2+} ionophore. This differs from other early growth factor–responsive genes, such as c-*fos* and *KC*, whose induction by these agonists is blocked by the identical downregulation protocol (19, 38). Thus, the regulation of TF in VSMC appears to be distinct from other early growth factor–inducible genes. These results raise the possibility that protein kinase C is not involved in agonist-mediated induction of TF mRNA. However, it is also possible that the downregulation protocol used in this study, while significantly reducing total protein kinase C activity, failed to block the activity of a specific protein kinase C isoform (39) responsible for the induction of TF mRNA. For example, Pettersen et al. (40), have recently shown that while phorbol ester–mediated downregulation of protein kinase C activity did not block the induction of TF mRNA by interleukin-1 or lipopolysaccharide in human umbilical vein endothelial cells, high doses of specific inhibitors of protein kinase C, such as H7 or staurosporine, did. While preliminary observations (Taubman, M., J. Marmur, and Y. Nemerson) suggest that specific protein kinase C inhibitors, such as H7, do not block the induction of TF mRNA, more detailed analysis will be required to more fully assess the role of protein kinase C in TF induction.

The early induction of TF in arterial VSMC is particularly provocative because of the potential role these cells play in the response to vascular injury. In experimental and clinical models of percutaneous balloon angioplasty, the endothelium is denuded, exposing the medial VSMC to circulating blood and thus to a variety of agents, such as PDGF, fibroblast growth factor, angiotensin II, and α-thrombin, all apparently capable of inducing TF expression. In addition, the VSMC are exposed to coagulation factors which upon interaction with TF can initiate the clotting cascade. Thrombus formation is an early event following vessel injury (41, 42) and may result in a rapid and total occlusion of the vessel lumen. This can largely be prevented by the use of platelet inhibitors and anticoagulants (43). Such treatment, while effective in preventing occlusive thrombosis, does not abolish the development of nonocclusive mural thrombus (44). This nonocclusive mural thrombus may play a critical role in the development of intimal hyperplasia by providing a foundation upon which platelets may aggregate and release growth factors. In addition, thrombin is a mitogen for cultured VSMC (45) and may play a role in stimulating proliferation in vivo.

The induction of TF in VSMC may be critical to the formation and/or propagation of the platelet-thrombus in the early phases of injury. The induction of TF by α-thrombin is particularly intriguing in that it demonstrates that a protein which is produced during activation of the coagulation cascade is capable of rapidly and markedly inducing mRNA encoding an initiator of this cascade. This raises the possibility that a positive feedback system exists whereby α-thrombin can help propagate the clot by inducing TF production in the VSMC located near the site of the thrombus. The findings in VSMC culture should serve as a stimulus to examine the expression of TF in VSMC in intact blood vessels under a variety of pathologic and physiologic conditions.

Acknowledgments

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