NF1 Regulatory Element Functions as a Repressor of Tissue Plasminogen Activator Expression

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Objective—Analysis of the distribution of endothelial cell tissue plasminogen activator (tPA) in the vasculature of rodents and primates demonstrated that tPA is constitutively expressed predominately in small artery endothelial cells of brain and lung. The regulatory elements responsible for the highly selective expression of arterial endothelial cell tissue plasminogen activator were sought.

Methods and Results—Transcription factor binding sites were defined by electrophoretic mobility-shift assay (EMSA) analysis using rat lung and brain nuclear extracts and the tPA promoter sequence from −609 to +37 bp. Protein binding to the promoter was found to be mediated by an NF1 site between −158 and −145 bp upstream from the transcriptional start site. Specific binding was confirmed through mutational analysis and competition binding studies. Infection of endothelial cells with a tPA promoter-green fluorescent protein (GFP) (−609 to +37 bp) reporter construct resulted in expression of the GFP, whereas no expression was found in smooth muscle cells. Mutation of the NF1 site increased the GFP expression indicating that the element acts as a repressor.

Conclusions—These results suggest that the 600 bp of the tPA promoter upstream of the transcription start site conveys cell specificity to tPA expression and that an NF1 site within this region acts as a repressor. (Arterioscler Thromb Vasc Biol. 2004;24:982-987.)

Key Words: endothelial cells ■ tissue plasminogen activator ■ gene expression ■ promoter ■ NF1

The endothelium is a functionally heterogeneous group of cells that respond to the diverse needs of specific tissue and organ environments, both constitutively and in response to changing physiological and pathologic conditions. Little is known about the origins of phenotypic diversity of endothelial cells, although inheritance from distinct sublineages (clonality) and microenvironmental effects have been proposed. The role of environmental factors promoting specific endothelial phenotypes involves the interaction of endothelial cells with other cell types such as astrocytes, smooth muscle cells, and myocytes, as well as the hemodynamic forces within the vessel lumen. These factors promote gene transcription of specific endothelial cell genes leading to unique endothelial cell phenotypes.

One protein whose expression is highly regulated in endothelial cells is tissue plasminogen activator (tPA). tPA expression occurs in a selective subset of endothelial cells that are defined by both vessel type and organ. The production of tPA and its secretion into the vasculature has been long assumed to be the responsibility of all endothelial cells. However, studies have demonstrated that in vivo expression of tPA is restricted to a distinct minority of endothelial cells. Immunohistochemical and in situ hybridization analysis of several large vessels in the nonhuman primate showed tPA antigen and mRNA exclusively localized to the endothelium of the vasa vasora, with none found in the large vessel endothelium. Physiological studies have indicated that tPA also is found in the brachial and coronary arteries. When a more thorough analysis of tPA distribution was performed in rodents, endothelial cell tPA expression was found to be predominately localized to the pulmonary arterial endothelium and the pia mater of the brain. This suggests that constitutive tPA expression is a characteristic of specific endothelial cells.

There have been numerous studies on the molecular regulation of tPA. Some of the experiments involved promoter mapping studies using various types of cultured cells, whereas others have used transgenic animals in which various lengths of promoter were inserted and site specific reporter-gene expression documented. Studies on endothelial cell regulation have been performed primarily with cultured cells from sources that do not express the protein in vivo. Because endothelial cells from most sources begin to express tPA when placed in culture it is not clear whether the results from the studies performed in vitro represent the mechanisms used in the intact animal. In the present study, we have characterized the tPA 5’ flanking region for elements that promote constitutive tPA expression within the endothelial cells of the brain and lung arterioles using freshly prepared tissues. We have examined the first 600 bp upstream from the
transcriptional start site, a sequence that contains a region of homology between the rat, mouse, and human promotor, for protein binding activity and have identified the participation of an NF-1 consensus sequence.

**Methods**

**Nuclear Extract Preparation and EMSA**

Nuclear extracts were prepared from the brains and lungs of 15-day-old rats (Harlan Breeders, Indianapolis, IN). Animals were anesthetized, decapitated, and the brains, lungs, kidneys, and livers removed and placed in buffer A (10 mmol/L HEPES, pH 7.9, 25 mmol/L KC1, 1 mM EGTA, 1 mmol/L EDTA, 0.32 mol/L sucrose, 0.15 mmol/L Spermine, 0.5 mmol/L spermidine, 1 mmol/L DTT, 0.5 mmol/L PMSF, 0.1 mmol/L Benzamidine, 1X complete protease inhibitor cocktail [Roche Molecular Biochemicals]), finely minced, and the volume of buffer A increased to a final volume equaling 5× the tissue weight. The samples were homogenized and the homogenate diluted 2 times in buffer A containing 2 mol/L sucrose. This mixture was layered onto undiluted buffer A containing 2 mol/L sucrose and the nuclei isolated by centrifugation for 45 minutes at 70,000g at 4°C. The nuclear pellet was washed twice in 600 μL of buffer A containing 20% glycerol and homogenized. To extract the nuclear proteins, 400 μL of buffer A containing 20% glycerol and 1 mol/L KC1 was added to the nuclear homogenate and the mixture incubated for 1 hour at 4°C with rocking. The samples were centrifuged at 244 587g for 50 minutes at 4°C. Nuclear extracts were partially purified by ion exchange chromatography using DEAE-Sephalic. Fractions containing eluted protein were dialyzed against 10 mmol/L Tris-HCl, pH 7.5, 50 mmol/L NaCl, 1 mmol/L MgCl2, 4% glycerol, 0.5 mmol/L EDTA, 0.5 mmol/L EGTA, 0.5 mmol/L DTT, 0.15 mmol/L Spermine, 0.5 mmol/L Spermidine, 0.5 mmol/L PMSF, 1 mM Benzamidine, concentrated, and stored at −80°C.

To identify potential binding sites within the promoter —609 to +37, oligonucleotide probes representing 4 overlapping regions of ~300 base pairs each were generated by polymerase chain reaction (PCR)-labeled on the 5’ end with biotin. The oligonucleotide probes were added at final concentration of 20 fmol to the reactions mixture containing 10 mmol/L Tris, pH 7.5, 50 mmol/L KC1, 1 mmol/L MgCl2, 4% glycerol, 0.5 mmol/L EDTA, 0.5 mmol/L EGTA, 0.5 mmol/L DTT, 0.15 mmol/L Spermine, 0.5 mmol/L Spermidine, 0.5 mmol/L PMSF, 1 mM Benzamidine, concentrated, and stored at −80°C. To determine potential binding sites within the promoter —609 to +37, oligonucleotide probes representing 4 overlapping regions of ~300 base pairs each were generated by polymerase chain reaction (PCR)-labeled on the 5’ end with biotin. The oligonucleotides were added at final concentration of 20 fmol to the reactions mixture containing 10 mmol/L Tris, pH 7.5, 50 mmol/L KC1, 1 mmol/L MgCl2, 4% glycerol, 0.5 mmol/L EDTA, 0.5 mmol/L EGTA, 0.5 mmol/L DTT, 0.15 mmol/L Spermine, 0.5 mmol/L Spermidine, 0.5 mmol/L PMSF, 1 mM Benzamidine, concentrated, and stored at −80°C.

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**Protein Binding Activity**

Comparison of the rat,19,20 mouse,15,21 and human22 tPA promoter sequence shows a high degree of homology within the first 300 bp proximal to the transcriptional start sites (Figure 1). The protein binding activity of a 600 bp fragment (−609 to +37) of the rat tPA promoter was examined by EMSA analysis using nuclear extracts derived from rat brain or lung. In the brain, both endothelial cells and neurons express tPA,10,11,23 whereas in the lung, endothelial cells are the only source of this protein.10,11 Therefore, to maximize the likelihood that endothelial cell-specific elements were being identified, only results that were identical with both lung and brain extracts were pursued. The initial EMSA analysis was performed with oligonucleotides representing 4 overlapping promoter fragments of −300 bp each. Specific binding was observed with fragment 1 (−287 to +37) and fragment 2.
To determine the sequence to which the proteins bound, fragment 1 was divided further into increasingly smaller overlapping oligonucleotides. The upper band observed in the 300-bp fragment disappeared as the fragments became smaller while the lower band remained, regardless of probe size. EMSA analysis showed complex formation with a 33-bp oligonucleotide probe representing the sequence between −165 and −132 (Figure 2C). Within this oligonucleotide exists an NF1 transcription factor consensus binding sequence.

Evidence that the NF1 site was specifically responsible for the protein binding was sought by performing EMSA analysis with 3 25-bp overlapping fragments containing either the complete or a partial NF1 binding sequence (Figure 3A). Only the fragment containing the complete NF-1 consensus sequence showed binding activity in both lung and brain. Neither kidney (Figure 3A) nor liver (data not shown) displayed specific binding of a protein to the probe indicating that the DNA–protein interaction was not a common occurrence among the various organs. To determine whether the protein binding region represented the NF1 consensus sequence, competition binding studies and mutational analysis were performed. The NF1 consensus sequence was mutated at positions critical for the recognition by NF1 (Figure 3B) and the mutated oligonucleotide analyzed by EMSA. No complexes were formed between the mutated NF1 sequence in either the lung or brain extracts. Binding could also be eliminated by competition with the complete NF1 sequence. Competition for binding with fragments a or c (sequence shown in Figure 3A) that contained partial NF1 consensus sequences had no effect on the binding activity of fragment b indicating that the intact sequence was necessary for protein binding.

**Functional Role of the NF1 Consensus Sequence**

To determine whether the promoter fragment studied can support transcriptional activity in cells that express tPA endogenously, adenoviral constructs containing the tPA promoter sequence −609 to +37 placed upstream from the coding sequence of GFP were added to cultures of HeLa cells, dermal microvascular cells, umbilical vein endothelial cells, and GFP expression evaluated (Figure 4). Photomicrographic analysis shows that GFP is expressed at detectable levels in each of these cells within 48 hours after infection. Infection of B109 cells, a neuroendocrine cell line, showed no GFP expression in the presence of the tPA promoter but were positive when the CMV promoter was substituted (data not shown). Mock infection controls were blank. A more detailed analysis of tPA promoter-driven GFP expression was performed by FACS using the 600 bp promoter-GFP (−609 to +37) construct, a shorter promoter sequence containing sequence −289 to +37, and each of these with a mutation residing in the NF-1 site (Figure 5). In endothelial cells, the lowest level of GFP expression was observed with the 600-bp fragment. A significant increase in expression (2.8-fold) occurred when the NF1 site was mutated (as in Figure 3C). Reduction in the length of the promoter to include the sequence from −287 to +37 also resulted in an increase in GFP expression even though the NF1 site was intact. A further increase in GFP expression was observed when the NF1 site was altered in this shortened fragment substantiating the results with the 600 bp promoter. When these studies were repeated with smooth muscle cells, no GFP expression was observed with any of the constructs despite the fact that cells infected with virus containing the CMV in place of the tPA promoter expressed as efficiently as the endothelial cells. In HeLa cells, GFP expression followed the same pattern as with the endothelial cells, with an increase when the NF1 site was mutated and when the fragment was shortened to 300 bp.

**Discussion**

In this study, we examined the possibility that regulatory elements within the tPA promoter region are involved with the control of limited endothelial cell tPA expression. Using a fragment consisting of the promoter sequence spanning −609 to +37, we performed EMSA analysis with nuclear fractions derived from brain and lung. The rationale for choosing these 2 organs is the presence of tPA producing endothelium, a condition that is absent from other organs analyzed. Fifteen-day-old animals were used because of the continued development of the lung and brain vasculature with the attendant proliferation of arterial endothelial cells. Such development would require continued transcriptional activity of the tPA gene. Within this fragment there appeared a protein binding activity that resided in an NF1 consensus sequence within 200-bp of the transcriptional start site. Commensurate with its protein binding activity is the repression of GFP expression as demonstrated by an increase in GFP expression in both endothelial and HeLa cells when the NF1 was altered. Cell specificity was suggested because smooth muscle cells and neuroendocrine cells infected with the virus containing the wild-type promoters had no detectable GFP expression. These findings are consistent with our hypothesis that the tPA promoter is responsible for regulating tPA expression in a cell type-dependent manner. This is also
supported by the absence of specific binding in kidney and liver, neither of which have detectable levels of endothelial cell tPA expression. Parallel results between endothelial cells and HeLa cells also were reported in other studies using plasmids containing promoter-reporter gene constructs containing 410 bp of the proximal promoter region. These constructs were sufficient to generate detectable levels of basal reporter activity in transfected endothelial cells and

Figure 2. Analysis of protein binding activity of the tPA promoter. A, The 4 overlapping fragments with which EMSAs were performed. B, EMSA analysis of the oligonucleotides depicted in (A). The DNA fragments were biotin-labeled and used in gel shift assays with lung or brain nuclear extracts from 15-day-old rats. Lane 1 represents the EMSA in the absence of extract. Lane 2 represents reactions with oligonucleotides and extract in the absence of competitor. Competitor nucleotides at 100-fold excess were the identical sequence (lane 3) or a nonhomologous sequence of identical length representing another portion of the promoter (lane 4). Each gel shift is representative of at least 3 separate studies with different preparations of nuclear extract. C, EMSA analysis of smaller fragments of Frag 4 showed protein binding within a 34 bp sequence −165 to −132. Lane 1, no extract; lane 2, 2.3 μg nuclear extract; lane 3, unlabeled specific competitor (100-fold); lane 4, nonspecific competitor (100-fold).

Figure 3. An NF1 site is responsible for protein binding. A, Oligonucleotides representing the tPA promoter fragment (−161 to −137) containing the NF1 binding site (denoted as fragment 2) and overlapping fragments that contain a partial NF1 sequence −173 to −149 (fragment 1) and −152 to −128 were generated and protein binding activity tested by EMSA as described. Lane 1, no extract; lane 2, 2.3 μg nuclear extract; lane 3, unlabeled specific competitor (100-fold excess); lane 4, nonspecific competitor (100-fold). B, Competition binding experiments between the three oligonucleotides shown in 3A and sequence −165 to −132 were performed to assess the role of flanking regions of the NF1. Gel shift assay reaction mixtures contained biotin-labeled oligonucleotide −165 to −132 and 100-fold excess of a, b, or c, or an NF1 consensus sequence 5′-TTTGGATTGAAGCCAATATGATAA-3′. C, The specificity of the NF1 site in protein binding was evaluated by site directed mutagenesis. Base substitutions are shown in italics and the NF1 sequence is underlined. EMSA analysis was then performed as described.
HeLa cells and in both cases were responsive to phorbol esters. HeLa cells produce endogenous tPA in culture and studies of the regulation of tPA antigen expression show a pattern consistent with endothelial cell regulation.24–26 An unexpected result in these studies was the increase in repressor activity when the portion of the promoter containing sequence /H11002 to /H11002 was removed from the construct. With this shorter construct, GFP expression increased considerably whether the NF1 was mutated. This region has no known regulatory elements and binding assays of fragment 2 and 3 (Figure 2) that contain all or part of this region show no bands that are specific to both lung and brain. These data suggest that the presence of this promoter fragment enhances the repressor activity of the NF1 through secondary effects, eg, by altering the structure of the DNA to allow for increased function at the NF1 site or that a factor exists within the cultured cells that is not present/detectable in tissues by EMSA.

The NF1 family of nuclear regulatory factors can both activate and repress gene transcription.27,28 In addition, NF1 can repress a promoter in 1 type of cell whereas activating the same promoter in another.29 Transcriptional repressors regulate gene expression by at least 2 distinct mechanisms. Nuclear proteins may compete with positive transcriptional activators for common DNA-binding sites, preventing binding of such factors, or destabilizing bound factors.30 They may also contain intrinsic repressor activity and the capacity to downregulate transcription through internal domains. It is not clear in the case of the tPA promoter whether the NF1 binding protein is acting passively or actively. However, the proximity of the NF1 site to a CRE (Figure 1) creates the opportunity for interaction between the 2 factors, an event that has been observed in other promoters.31–33 In addition to the NF1 site, other regulatory elements within the first 200 bp of the proximal promoter region have been associated with regulation of constitutive t-PA expression as well as induction of expression in response to various agonists.15,20,21,24,27,34,35 In HeLa cells, a cAMP response-like element and an SP-1 site act cooperatively to mediate phorbol-ester-mediated induction of tPA gene expression, and in endothelial cells cyclic

**Figure 4.** Functional analysis of the tPA promoter fragment. tPA promoter fragment −609 to +37 was placed upstream of the GFP gene and the construct inserted into an adenoviral vector. Cells were infected with the virus containing either the tPA promoter or a CMV promoter at 20 pfu/cell, and 48 hours later the cultures were analyzed. A, dermal microvascular endothelial cells; B, HeLa cells. C, venous endothelial cells. Upper panel, cells infected with the tPA promoter adenoviral constructs showing GFP expression; middle panel, Brightfield photomicrographs of the corresponding cultures; lower panel, cells infected with adenovirus containing the CMV promoter driving GFP expression.

**Figure 5.** GFP expression under the control of the tPA promoter. A, FACS analysis of GFP expression in endothelial cells. Endothelial cells were infected with adenoviral constructs containing the tPA promoter encompassing bases −609 to +37 (panel 1), −289 to +37 (panel 2), −609 to +37 with base substitutions in the NF1 site (panel 3), and −289 to +37 with the same mutations in the NF1 site (panel 4). Base substitutions are the same as those shown in Figure 3C and eliminate protein binding to the site. All cells were infected at 50 pfu/cell with either viral constructs containing the tPA promoters or CMV promoter. The construct used in panel 1 (−609 to +37) correlates to the conditions used in Figure 4. B, Comparison of GFP expression in endothelial cells, smooth muscle cells, and HeLa cells infected with the various wild-type and mutant promoter constructs described. Data are presented as a percent of the number of cells infected with CMV-GFP viral construct and which are expressing GFP. Endo indicates endothelial cells; smc, smooth muscle cells.
strain induction was shown to be dependent on the CRE and an AP-2 site. In summary, this study identifies a binding sequence in the tPA promoter that binds to an as yet unidentified protein found in the nuclear extract of lung and brain. This protein acts as a repressor of tPA expression in endothelial cells and other cells that produce tPA in vitro. In addition, promoter activity is absent in smooth muscle cells that are incapable of producing tPA in vivo.

References

1. Augustin HG, Kozian DH, Johnson RC. Differentiation of endothelial cells: Analysis of the constitutive and activated endothelial cell phenotypes. Bioessays. 1994;16:901–906.
2. Goerd S, Sorg C. Endothelial heterogeneity and the acquired immunodeficiency syndrome: A paradigm for the pathogenesis of vascular disorders. Clin Invest. 1992;70:89–98.
3. Masawa T, Kawasaki DA. Retroviral analysis of cardiac morphogenesis: discontinuous formation of coronary vessels. Proc Natl Acad Sci U S A. 1992;89:9504–9508.
4. Wang R, Clark R, Bauch VL. Embryonic stem cell-derived cystic embryoid bodies form vascular channels: an in vitro model of blood vessel development. Development (Camb). 1992;114:303–316.
5. Resnick N, Gimbonte MA. Hemodynamic forces are complex regulators of endothelial cell gene expression. FASEB J. 1995;9:874–882.
6. Lobrinus JA, Juillerat-Jeanneret L, Darekar P, Schlosshauer B, Janzer RC. Induction of the blood brain barrier specific H1T and neurotransmitter epitopes in endothelial cells of the chick choroidallantoic vessels by a soluble factor derived from astrocytes. Brain Res Dev Brain Res. 1992;70:207–211.
7. Nishida M, Springhorn JP, Kelly RA, Smith TW. Cell-Cell signaling between adult rat venricular myocytes and cardiac microvascular endothelial cells in heterotypic primary culture. J Clin Invest. 1993;91:1934–1941.
8. Ard WC, Edelberg JM, Weiler-Guettler H, Simmons WW, Smith TW, Rosenberg RD. Vascular bed-specific expression of an endothelial cell gene is programmed by the tissue microenvironment. J Cell Biol. 1997;138:1117–1124.
9. Levin EG, Santell L, Osborn KG. The expression of tissue plasminogen activator in vivo: a function defined by vessel size and anatomic location. J Cell Sci. 1997;110:139–148.
10. Levin EG, Banka CL, Parry GC. Progressive and transient expression of tissue plasminogen activator during fetal development. Arterioscler Thromb Vasc Biol. 2000;20:1668–7420.
11. Levin EG, del Zoppo GJ. Localization of tissue plasminogen activator in the endothelium of a limited number of vessels. Am J Pathol. 1994;144:855–861.
12. Dovjak N, Blinc A, Keber D. In vivo release of tissue-type plasminogen activator antigen from the human brachial artery. Arterioscler Thromb Res. 2002;106:249–255.
13. Osterlund B, Andersson B, Haggmark S, Jern C, Johansson G, Seeman-Lodding H, Biber B. Myocardial ischemia induces coronary t-PA release through its multihormone-responsive enhancer. FEBS Lett. 1999;460:289–296.
14. Feng P, Ohlsson M, Ny T. The structure of the TATA-less rat tissue-type plasminogen activator gene. Species-specific sequence divergences in the promoter predict differences in regulation of gene expression. J Biol Chem. 1990;265:2022–2027.
15. Ohlsson M, Leonardsson G, Jia XC, Feng P, Ny T. Transcriptional regulation of the rat tissue type plasminogen activator gene: Localization of DNA elements and nuclear factors mediating constitutive and cyclic AMP-induced expression. Mol Cell Biol. 1993;13:266–275.
16. Dzabow AL, Rickles RF, Pollock AE, Silly G, Di S, Tr. The transcription factor Sp1 is important for retinoic acid-induced expression of the tissue plasminogen activator gene during F9 teratocarcinoma cell differentiation. Mol Cell Biol. 1990;10:5883–5893.
17. Degen SJ, Reich E. Human tissue plasminogen activator gene. J Biol Chem. 1986;261:6972–6985.
18. Strickland S. Tissue plasminogen activator in nervous system function and dysfunction. Thromb Haemost. 2001;86:138–143.
19. Costa M, Shen Y, Maurer F, Medcalf RL. Transcriptional regulation of the tissue-type plasminogen-activator gene in human endothelial cells: identification of nuclear factors that recognise functional elements in the tissue-type plasminogen-activator gene promoter. Eur J Biochem. 1998;258:123–131.
20. Allen EH, Hamilton JA, Medcalf RL, Kubota M, Martin TJ. Cyclic AMP-dependent and independent effects on tissue-type plasminogen activator in osteogenic sarcoma cells; evidence from phosphodiesterase inhibition and parathyroid hormone antagonists. Biochim Biophys Acta. 1986;888:199–207.
21. Medcalf RL, Rieggl M, Schleuning W-D. A DNA motif related to the cAMP-responsive element and an exon-located activator protein-2 binding site in the human tissue-type plasminogen activator gene promoter cooperate in basal expression and convey activation by phorbol ester and cAMP. J Biol Chem. 1990;265:14618–14626.
22. Szabo P, Moitra J, Rencendorj A, Rakhely G, Rauch T, Kiss I. Identification of a nuclear factor I family protein-binding site in the silenced region of the cartilage matrix protein gene. J Biol Chem. 1995;270:10212–10221.
23. Adams AD, Choate DM, Thompson MA. NF1-L is the DNA-binding component of the protein complex at the peripherin negative regulatory element. J Biol Chem. 1995;270:6975–6983.
24. Gao B, Kunos G. Cell type-specific transcriptional activation and suppression of the alpha1B adrenergic receptor gene middle promoter by nuclear factor I. J Biol Chem. 1998;273:31784–31787.
25. Gronostajski RM. Roles of the NF1/CFL family gene in transcription and development. Gene. 2000;249:31–45.
26. Leahy P, Crawford DR, Grossman G, Gronostajski RM, Hansom RW, CREB binding protein coordinates the function of multiple transcription factors including nuclear factor K family protein-binding site in the silencer region of the cartilage matrix protein gene. J Biol Chem. 1995;270:808–812.
27. Ortiz L,Aza-Blanc P, Zannini M, Cato AC, Santisteban P. The interaction between the forkhead thyroid transcription factor TTF-2 and the constitutive factor CTIF/NF-1 is required for efficient hormonal regulation of the thyroxoperoxidase gene transcription. J Biol Chem. 1999;274:8815–8822.
28. Lanet MA, Pouyer GG, Guerin SL. Nuclear factor 1 interferes with Sp1 binding through a composite element on the rat poly(ADP-ribose) polymerase promoter to modulate its activity in vitro. J Biol Chem. 2001;276:20766–20773.
29. Leonardsson G, Ny T. Characterisation of the rat tissue-type plasminogen activator gene promoter—identification of a TAAT-containing promoter element. Eur J Biochem. 1997;248:676–683.
30. Yu H, Schleuning WD, Michl M, Liberte A, Tan SS, Medcalf RL. Control elements between -9.5 and -3.0 kb in the human tissue-type plasminogen activator gene promoter direct spatial and inducible expression to the murine brain. Eur J Neurosci. 2001;14:799–808.
31. Sumpio BE, Chang R, Xu WJ, Wang XJ, Du W. Regulation of Ipa in endothelial cells exposed to cyclic strain: role of CRE, AP-2, and SSRE binding sites. Am J Physiol. 1997;273:C1441–C1448.