Arginine 260 of the Amino-terminal Domain of NR1 Subunit Is Critical for Tissue-type Plasminogen Activator-mediated Enhancement of N-Methyl-D-aspartate Receptor Signaling*

Mónica Fernández-Monreal‡‡‡, José P. López-Atalaya‡‡‡, Karim Benchenane‡‡‡‡, Mathias Cacquevel‡, Fabienne Dulin‡, Jean-Pierre Le Caer‡‡‡, Jean Rossier§§§, Anne-Charlotte Jarrige‡, Eric T. MacKenzie‡, Nathalie Colloch’À, Carine Ali‡, and Denis Vivien‡‡‡

From the ¶¶CNRS UMR 6185, University of Caen, Centre Cytéeron, Bd. Henri Becquerel, BP 5229, 14074, Caen cedex, France, ‡École Polytechnique CNRS UMR 7651, 91281 Palaiseau Cedex, France, and §§ESPCI, CNRS UMR 7637, 75231 Paris cedex 5, France

Tissue-type plasminogen activator (tPA) has been involved in both physiological and pathological glutamatergic-dependent processes, such as synaptic plasticity, seizure, trauma, and stroke. In a previous study, we have shown that the proteolytic activity of tPA enhances the N-methyl-D-aspartate (NMDA) receptor-mediated signaling in neurons (Nicole, O., Docagne, F., Ali, C., Margail, I., Carmeliet, P., MacKenzie, E. T., Vivien, D., and Buisson, A. (2001) Nat. Med. 7, 59–64). Here, we show that tPA forms a direct complex with the amino-terminal domain (ATD) of the NR1 subunit of the NMDA receptor and cleaves this subunit at the arginine 260. Furthermore, point mutation analyses show that arginine 260 is necessary for both tPA-induced cleavage of the ATD of NR1 and tPA-induced potentiation of NMDA receptor signaling. Thus, tPA is the first binding protein described so far to interact with the ATD of NR1 and to modulate the NMDA receptor function.

This work was supported by grants from the CNRS, University of Caen and European Council (FEDER). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

To whom correspondence should be addressed. Tel.: 33-231-56-60-39; Fax: 33-231-56-61-99; E-mail: d.vivien@neuro.unicaen.fr.

* This work was supported by grants from the CNRS, University of Caen and European Council (FEDER). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† Supported by the Fondation pour la Recherche Medicale.
‡ Supported by the Regional Council of Lower Normandy.
§ Supported by the Ministère de l’Education Nationale, de l’Enseignement Supérieur et de la Recherche.

The abbreviations used are: tPA, tissue-type plasminogen activator; LTP, long term potentiation; L-LTP, late phase LTP; NMDA, N-methyl-D-aspartate; ATD, amino-terminal domain; HEK, human embryonic kidney; MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight; LIVBP, leucine-isoleucine-valine-binding protein; tPA-Stop®, 2,7-bis-(4-aminobenzydilene)-cyloheptan-1-one dihydrochloride.

** This work was supported by grants from the CNRS, University of Caen and European Council (FEDER). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

†† Supported by the Fondation pour la Recherche Medicale.
‡‡ Supported by the Regional Council of Lower Normandy.
§§ Supported by the Ministère de l’Education Nationale, de l’Enseignement Supérieur et de la Recherche.

Materials—Horse serum and fetal bovine serum were purchased from Invitrogen. L(+)-amino-5-phosphonopentanoic acid was from Tocris (Bristol, United Kingdom). Human recombinant tPA was purchased from Boehringer Ingelheim (Paris, France). α-Casein was obtained from

EXPERIMENTAL PROCEDURES

This paper is available on line at http://www.jbc.org
tPA Is a New Ligand of NMDA Receptor

ICN Biomedicals (Aurore, OH), and human Lys-plasminogen was purchased from Santa Cruz Biotechnology (Heidelberg, Germany) and Qiagen (Courtaboeuf, France), respectively. 2,7-Bis-(4-amidinobenzylidene)-cycloheptan-1-one dihydrochloride (H11003) and tPA substrate spectrozyme XF-444 were purchased from Calbiochem. Antibodies raised against the NR1 subunit were revealed with an antibody raised against His6 (H9262).

Cortical Cultures—Neuronal cortical cultures were prepared from fetal mice (embryonic day 15–16). Dissociated cortical cells were resuspended in Dulbecco’s modified Eagle’s medium supplemented with 5% fetal bovine serum, 5% horse serum, and 2 mM glutamine and plated in 24-well dishes previously coated with poly-D-lysine and laminin. After 3 days in vitro, the cells were exposed to 10 μM Ara-C to inhibit glial proliferation. Cultures were used after 14 days in vitro (30).

Immunoblotting—Protein samples were resolved on SDS-polyacrylamide gel and transferred onto a polyvinylidene difluoride membrane. Membranes were blocked with 5% dried milk in Tris-buffered saline containing 0.05% Tween 20 and incubated with primary antibodies. After incubation with the corresponding biotinylated secondary antibody and peroxidase-conjugated streptavidin reagent, proteins were visualized with an enhanced chemiluminescence ECL Plus immunoblotting detection system (PerkinElmer Life Sciences).

Human Embryonic Kidney (HEK)-293 Cell Cultures and Transient Transfection—Human embryonic kidney 293 cells (ATCC 1573-CRL) were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. Low confluency cells were transfected by the calcium phosphate precipitation method (31) with a mixture containing NR1–1a/b (or NR1–1aR260A), NR2A, and enhanced green fluorescent protein plasmids (1, 1, and 0.3 μg/coverlisp, respectively). After transfection, NMDA antagonists (200 μM AP5, 2 mM MgCl2, 1 mM kynurenic acid) were added to the culture medium. Experiments were performed within 36–48 h after transfection.

Calcium Videomicroscopy Analysis—Transfected HEK-293 cells were loaded in the presence of a HEPES-buffered saline solution containing 5 μM fur-2/AM plus 0.1% pluronic F-127 (Molecular Probes, Leiden, the Netherlands) (30 min, 37 °C) and incubated for an additional 30-min period in a HEPES-buffered saline solution. Experiments were performed at room temperature on the stage of a Nikon Eclipse inverted microscope equipped with a 75 W Xenon lamp and a Nikon 40×, 1.3 numerical aperture epifluorescence oil immersion objective. Fura-2 (excitation 340 and 380 nm, emission 510 nm) ratio images were acquired with a CCD camera (Princeton Instrument, Trenton, New Jersey) and digitized (256 × 512) using Metamorph 4.11 software (Universal Imaging Corporation, Chester, Pennsylvania).

Construction of His-tagged Recombinant ATD/Leucine-Isoleucine-Valine-binding Protein (LIVBP)-like Domain—The region of the NR1 subunit encoding amino acids 19–371 for NR1–1a or 19–389 for NR1–1b corresponding to the ATD was amplified from the full-length rat NR1–1a or NR1–1b cDNA, respectively, by using the upstream primer 5-CCGGATCCCGGCGCCCGCCCGGCGAC-3 generating a BamHI restriction site and the downstream primer 5-ATGGGTACCATTGTAGATGAC-3', containing an internal KpnI restriction site. PCR products were digested and inserted in pQE100-Double Tag vector (Qiagen), which encodes for His6 at the amino terminus of the insert. Recombinant proteins were purified from inclusion bodies of isopropyl 1-thio-β-D-galactopyranoside-induced bacterial cultures (Escherichia coli, M15 strain) on a nickel affinity matrix as described by the manufacturer (Qiagen).

Site-directed Mutagenesis—Mutagenesis of either recombinant NR1–ATD (R260A and R217A) or full-length NR1–1a (R260A) was performed by using QuikChange® XL site-directed mutagenesis kit purchased from VWR International France (Fontenay-sous-Bois, France). All mutations were confirmed using an automated sequence analysis.

Enzymatic Assay—Recombinant tPA (29 nM) was incubated in the presence of a tPA-specific fluorogenic substrate (5 μM) (Spectrozyme®...
tPA Is a New Ligand of NMDA Receptor

We have previously shown that tPA potentiates NMDA receptor-mediated Ca\(^{2+}\) influx in cultured cortical neurons (1). To further investigate the mechanism of action of tPA on NMDA receptor signaling, we have examined whether tPA, per se, can cleave NR1. We have shown previously that the treatment of membrane preparations of cultured cortical neurons with tPA leads to the appearance of a cleaved form of NR1, with a molecular mass reduced to 25 kDa, recognized by an antibody raised against the carboxyl terminus of NR1 (1). This suggests that the amino-terminal portion of the NR1 subunit is the region in which the cleavage occurs. Hence, to determine where tPA cleaves the amino terminus of NR1, we have generated a construct encoding for an amino terminus His-tagged corresponding to the full-length NR1 (of neuronal membrane preparations from rat brain). Finally, as determined by the digestion pattern of the recombinant NR1-ATD generated by tPA or plasmin, we observed that although tPA induces a single cleavage of the ATD of NR1, plasmin leads to a complete degradation of the full-length NR1 (of neuronal membrane preparations from rat brain) and the recombinant NR1-ATD (Fig. 2A). Incubation of recombinant NR1-ATD with tPA shows that tPA is able to cleave NR1-ATD, in a dose-dependent manner, generating a cleaved fragment of 28 kDa detected by immunoblotting revealed with an antibody raised against the His-tag (Fig. 2B). After incubation of recombinant NR1-ATD with tPA, the ATD was subjected to acetonitrile washing and reductive alkylation by iodoacetamide. The sample was then analyzed by MALDI-TOF mass spectrometry (Fig. 2C). The data sets on the inhibition of proteases were obtained with a Voyager-DE STR Biospectrometry Work station mass spectrometer (PE Biosystems Inc.). Analyses were performed in positive ion reflector mode, with an accelerating voltage of 20,000 V, a delayed extraction of 200 ns, and ~250 scans were averaged. Subsequent data processing, the Data Explorer software (PE Biosystems Inc.) was used. Spectra obtained for the whole protein were calibrated externally using the [M+H]+ ion from Des-Arg bradykinin peptide (904.681 Da) and ACTH peptide (2450.1689 Da). The trypsin autoproteolysis products (fragment-(132–142), 1153.57 Da and fragment-(56–75), 2163.06 Da) were used as the external calibration standard. A mass deviation of 0.1 Da was allowed in the data base searches.

**RESULTS**

**tPA Potentiates NMDA Receptor-mediated Ca\(^{2+}\) Influx in HEK-293 Cells Expressing NMDA Receptors Containing Either NR1–1a or NR1–1b**—We have previously shown that tPA enhances the NMDA-evoked Ca\(^{2+}\) influx in cultured cortical neurons (1, 7). To investigate the molecular mechanism through which tPA is able to modulate the NMDA receptor signaling, we developed a heterologous expression system of functional NMDA receptors. We transiently transfected HEK-293 cells with the cDNA encoding for NR1–1a and NR2A subunits of the NMDA receptor. The expression of NMDA receptor subunits was confirmed by immunocytchemistry and immunoblotting analyses (data not shown). As shown in Fig. 1, in HEK-293 cells expressing NMDA receptors, tPA is able to potentiate NMDA receptor signaling, as it does in cortical neurons (1). The mRNA encoding for the NR1 subunit exhibits an alternative splicing in its 5’-region, generating isoforms characterized by the absence (a forms) or the presence (b forms) of a 21-residue insert (N1 cassette) encoded by exon 5 (37). Because the exon 5 splicing is known to influence receptor properties (38–41), we have assessed its potential implication in tPA effect. We found that tPA increases NMDA-induced Ca\(^{2+}\) influx to the same extent in HEK-293 cells exhibiting NR1–1b/NR2A or NR1–1a/ NR2A receptors (36.87 ± 10.9%, n = 32 and 31.37 ± 5.4%, n = 56, respectively, mean ± S.E.) (Fig. 1, A–C). Hence, the N1 cassette does not influence the tPA-induced potentiation of NMDA receptor signaling.

**tPA Induces a Single Cleavage of NR1 in Its ATD**—To further investigate the mechanism of action of tPA on NMDA receptor signaling, we have examined whether tPA, per se, can cleave NR1. We have shown previously that the treatment of membrane preparations of cultured cortical neurons with tPA leads to the appearance of a cleaved form of NR1, with a molecular mass reduced to 25 kDa, recognized by an antibody raised against the carboxyl terminus of NR1 (1). This suggests that the amino-terminal portion of the NR1 subunit is the region in which the cleavage occurs. Hence, to determine where tPA cleaves the amino terminus of NR1, we have generated a construct encoding for an amino terminus His-tagged corresponding to the first domain of NR1–1a (amino acid residues 19–371 of NR1, 39875 Da), termed ATD or LIVBP-like domain (Fig. 3A). In addition, tPA-Stop®, an inhibitor of tPA proteolytic activity, prevents the tPA-induced cleavage of both the full-length NR1 (of neuronal membrane preparations from cultured cortical neurons) and the recombinant NR1-ATD (Fig. 3A). Finally, as determined by the digestion pattern of the recombinant NR1-ATD generated by tPA or plasmin, we observed that although tPA induces a single cleavage of the ATD of NR1 subunit, plasmin leads to a complete degradation of the ATD (Fig. 3B).

**The ATD of NR1 Is a Substrate of tPA**—The next question was to determine whether the ATD of NR1 could be a direct substrate for tPA. To address this question, we have used the recombinant ATD of NR1 to compete the ability of tPA to cleave a specific fluorogenic substrate (Spectrozyme®, XF444). Our data showed that the recombinant ATD of NR1 is able to compete with the tPA-specific substrate with a Kₘ of 0.234 ± 0.097 µM (Table I). Control experiments were performed in
parallel in the presence of the tPA inhibitor tPA-Stop® with a $K_i$ of 0.046 ± 0.015 μM (Table I).

**tPA CLEAVES THE ATD OF NR1 AT THE ARGinine 260—** Next, to identify the exact location of the cleavage site, we analyzed both native (39 kDa) and tPA-cleaved (28 kDa) recombinant NR1-ATD using MALDI-TOF analysis. As summarized in Fig. 4, MALDI-TOF analysis allowed us to identify the putative cleavage site of the NR1–1a and NR1–1b subunits by tPA as the arginine in position 260 (Arg-260) and arginine 281 respectively (Fig. 4A). Sequence analyses revealed that the tPA-
cleft region “ISIGNALRYAPDG” is highly conserved in NR1 subunits of NMDA receptors, whatever the species analyzed, and not found in other NMDA receptor subunits (data not shown). The analysis of the homology model of the ATD of the NR1 subunit (Fig. 4, B and C) shows that the Arg-260 is located close to the entry of a hydrophobic pocket for which no ligand has been described so far.

**Mutation of the Arg-260 Prevents both tPA-induced Cleavage of NR1-ATD and Potentiation of NMDA-Receptor Signaling—**To validate mass spectrometry analyses, we performed a mutation of the Arg-260 (and Arg-217 as a negative control) of the NR1-ATD recombinant protein into alanine and tested the ability of tPA to cleave these proteins. Although tPA cleaved both wild-type and control R217A proteins, it failed to cleave the protein mutated at the arginine in position 260 (Fig. 5A). Moreover, wild-type NR1-ATD inhibited the ability of tPA to cleave its specific fluorogenic substrate (40%, p < 0.01). In contrast NR1R260A-ATD had no effect on tPA activity (Fig. 5B). Next, to determine whether a point mutation of this arginine at the position 260 could prevent the tPA-induced potentiation of NMDA receptor-mediated signaling, we transiently co-transfected HEK-293 cells with NR2A in the presence of either wild-type or R260A mutated NR1–1a, and performed calcium videomicroscopy experiments. When co-transfected with NR2A, both wild-type and R260A mutated NR1–1a subunits displayed the same responses to increasing concentrations of NMDA (Fig. 6, A and B). In contrast, although the addition of exogenous tPA potentiated NMDA-induced Ca^{2+} influx in HEK-293 cells co-transfected with wild-type NR1–1a and NR2A, tPA failed to potentiate NMDA receptor signaling in transfected cells containing the mutated NR1–1a R260A subunit and NR2A (27.57 ± 3.7%, n = 102 and 2.49 ± 1.7%, n = 110, respectively) (Fig. 7, A and B).

**DISCUSSION**

Fast excitatory neurotransmission in the mammalian central nervous system is mediated by ionotropic glutamate-gated receptors. When overstimulated, ionotropic glutamate-gated receptors, particularly the NMDA subtype, cause excitotoxicity. We have shown previously that the serine protease tPA potentiates NMDA receptor signaling, which might be of particular relevance in several physiological and pathological brain conditions (1). However, whether this effect is the direct consequence of the ability of tPA to bind to and then cleave the NMDA receptor NR1 subunit remained to be demonstrated. Here, we demonstrated that tPA cleaves NR1 within a particular extracellular region, the ATD, in which we identified the arginine in position 260 as a critical residue for the tPA-induced potentiation of NMDA signaling.

First, by using transfected HEK cells, we show that tPA potentiates NMDA signaling indifferently in receptors containing either NR1a or NR1b splice variants. Both isoforms differ by the absence or presence of a amino-terminal N1 cassette, known to modulate some properties of the NMDA receptor (38–41). Our results thus demonstrated that the N1 cassette is not involved in the potentiating activity of tPA on NMDA receptor signaling. We also demonstrated that tPA can interact with and cleave the ATD of both NR1a and b subunits. The ATD of NR1 also LIVBP-like domain is a conserved domain found in all metabotropic and ionotropic glutamate receptor subunits described so far. Mutagenesis analyses within the ATD of NMDA receptor subunits have suggested that this domain might regulate the functional features of this receptor, by controlling subunit-subunit interactions (42) and/or altering allosteric modulations (43–45). Our present data provide a novel function for the ATD of NR1. Indeed, we showed that this ATD of NR1 is a binding site for tPA and that the cleavage of its arginine 260 in NR1-a (arginine 281 for NR1-b) is a necessary event for tPA-induced potentiation of NMDA signaling.

As previously suggested (46, 47) whether tPA could form a direct complex with NR1 or through the participation of its classical substrate plasminogen remained to be determined. Although the presence of locally synthesized tPA in the central nervous system has been well characterized (3, 5) that of plasminogen is still a matter of debate (3, 5, 6, 48, 49). Hence, we can not exclude the idea that tPA could cleave the NMDA receptor via the activation of plasminogen into plasmin. Indeed, activated plasminogen has been shown to lead to a complete degradation of the NR1 subunit of NMDA isolated from brain lysates (47). Accordingly, we showed that plasmin leads to a complete degradation of the ATD of NR1. However, this proteolytic pattern differs from that of tPA, which induces a single cleavage at the Arg-260. Thus, these data demonstrated that tPA-induced cleavage of NR1 is not mediated by plasmin, which is in agreement with our previous observations that in contrast to tPA, plasmin does not influence NMDA receptor-mediated neuronal death (1).

Another important issue is to know whether the endogenous concentrations of tPA are high enough to lead to the cleavage of the NR1 subunit of the NMDA receptor and subsequent potentiation of NMDA-mediated signaling in vivo situations. In a previous study, we have shown that in cultured cortical neurons, NMDA receptor activation is sufficient to promote both tPA release and subsequent cleavage of the NR1 subunit of NMDA receptor. In addition, this NMDA-dependent cleavage of NR1 is inhibited by exogenous plasminogen activator inhibitor-1 and does not occur in tPA-deficient cortical neurons (1). Additionally, we have demonstrated that the blockade of the proteolytic activity of endogenous tPA reduces NMDA-induced excitotoxic death (1, 30). Nevertheless, the concentration of tPA

**FIG. 5.** Mutation of the Arg-260 into alanine prevents tPA-induced cleavage of the ATD of NR1 subunit. A, immunoblots (WB) were performed from both wild-type and mutated recombinant ATD-NR1 (R260A and R217A) incubated in the presence of tPA (20 μg/ml) and revealed with an antibody raised against His6. Induced cleavage of the ATD of NR1 subunit.

**TABLE 5.**

| tPA activity (A.U.) | + | + | + | + |
|--------------------|---|---|---|---|
| tPA                | + | + | + | + |
| tPA-NR1            | + | + | + | + |
| tPA-NR1R260A       | + | + | + | + |

**FIG. 6.** A, Western blots (WB) 6 h post-HS show cleaved fragments of ATD-NR1 subunits of NMDA receptors. B, histogram showing the ability of recombinant ATD-NR1 or recombinant ATD-NR1R260A to inhibit tPA amidolytic activity, assessed by the cleavage of Spectrozyme® XP444 (tPA = 29 nM, ATD-NR1 = ATD-NR1R260A = 225 nM).
and NR1–1aR260A/NR2A transfected cells (expressed receptors exhibit a dose-dependent NMDA-evoked calcium influx (pendent responses assessed by digital quantitation of $[Ca^{2+}]_i$). NMDA responses in HEK-293 cells transiently transfected with NR1–1a/NR2A (2.5 ± 3.86% (n = 5, n (cell number) = 56) or NR1–1aR260A/NR2A receptors (n = 2, n (cell number) = 47). Both heterologously expressed receptors exhibit a dose-dependent NMDA-evoked calcium influx (p < 0.0001), and no difference was observed between NR1–1a/NR2A and NR1–1aR260A/NR2A transfected cells (p > 0.56 by two-way analysis of variance followed by Bonferroni correction).

FIG. 6. Mutation of the Arg-260 into alanine does not influence basal NMDA receptor signaling. A, representative NMDA dose-dependent responses assessed by digital quantitation of $[Ca^{2+}]_i$, with fura-2 performed in HEK-293 cells transiently transfected with NR1–1a/NR2A (left panel) or NR1–1aR260A/NR2A receptors (right panel). B, data are the mean (± S.E.) of NMDA responses in HEK-293 cells transiently transfected with NR1–1a/NR2A (n = 2, n (cell number) = 56) or NR1–1aR260A/NR2A receptors (n = 2, n (cell number) = 47). Both heterologously expressed receptors exhibit a dose-dependent NMDA-evoked calcium influx (p < 0.0001), and no difference was observed between NR1–1a/NR2A and NR1–1aR260A/NR2A transfected cells (p > 0.56 by two-way analysis of variance followed by Bonferroni correction).

FIG. 7. Mutation of the Arg-260 into alanine prevents tPA-induced potentiation of NMDA receptor-evoked calcium influx. A, representative records of NMDA-evoked $[Ca^{2+}]_i$, show an increase prior to and after addition of tPA (20 μg/ml) to the perfusion solution for 10 min in HEK-293 cells transiently transfected with NR1–1a/NR2A (left panel) or NR1–1aR260A/NR2A receptors (right panel). B, tPA exposure enhances NMDA responses in NR1–1a/NR2A-transfected cells of 27.6 ± 3.86% (n = 5, n (cell number) = 102), whereas no potentiation is observed in cells expressing receptors containing R260A mutated NR1–1a subunit (2.5 ± 1.7%, n = 5, n (cell number) = 110). Data presented are the percentage of increase of NMDA-induced calcium influx (mean ± S.E.; *, indicates significantly different from stimulations before tPA application; #, indicates difference between NR1 and NR1R260A by one-way analysis of variance followed by a Bonferroni-Dunn’s test for multiple comparison (p < 0.0001)).

not only in the brain parenchyma but also at the synaptic cleft remain actually undetermined.

In physiological conditions, an impairment or increase of L-LTP has been observed in tPA-deficient or tPA-overexpressing mice, respectively (12–14). In addition to hippocampal functions, tPA has also been involved in phenomena such as cerebellar motor learning (15, 50), visual cortex plasticity (51), and fear conditioning (52). During the last several years, several reports have also demonstrated a connection between tPA and brain disorders. tPA has been involved in many pathological processes including stroke (21, 25), seizure (19, 20, 53), and multiple sclerosis (23, 24). For instance, tPA-deficient mice display a high resistance to neuronal damages induced through either excitotoxic paradigms (19, 24) or experimental ischemia (21, 25). Conversely, plasminogen activator inhibitor-1-deficient mice show an increased volume of infarct following cerebral ischemia (25). In addition, mice overexpressing neuroserpin, another tPA inhibitor, have a reduced volume of lesion following ischemic brain injury (54). Hence, these data should represent the basis of the development of new therapeutic strategies targeting the interaction between tPA and NMDA receptors.

Acknowledgments—We thank the CRIHAN, the Centre Européen de Bioprospectives, and the FEDER for the use of the visualization software Insight II (Accelrys, San Diego, CA).

REFERENCES

1. Nicole, O., Docagne, F., Ali, C., Margaill, I., Carmeliet, P., MacKenzie, E. T., Vivien, D., and Buissone, A. (2001) Nat. Med. 7, 59–64
2. Sumi, Y., Dent, M. A., Owen, D. E., Seeley, P. J., and Morris, R. J. (1992) Development 116, 625–637
3. Sappino, A. P., Madani, R., Huarte, J., Belin, D., Kiss, J. Z., Wohwend, A., and Vassalli, J. D. (1993) J. Clin. Invest. 92, 679–685
4. Carroll, P. M., Tsirka, S. E., Richards, W. G., Frohman, M. A., and Strickland, S. M. (1994) Development 120, 3173–3183
5. Davies, B. J., Pickard, B. S., Steel, M., Morris, R. G., and Rathe, R. (1998) J. Biol. Chem. 273, 23004–23011
6. Tsirka, S. E., Bogove, A. D., Bugge, T. H., Degen, J. L., and Strickland, S. (1997) J. Neurosci. 17, 543–552
7. Fernandez-Monreal, M., Lopez-Aalaya, J. P., Benchenane, K., Leveille, F., Cacquevel, M., Flawsinski, L., MacKenzie, E. T., Bu, G., Buissone, A., and Vivien, D. (2004) Mol. Cell Neurosci. 25, 584–601
8. Gualandris, A., Jones, T. E., Strickland, S., and Tsirka, S. E. (1996) J. Neurosci. 16, 2220–2225
9. Baranes, D., Lederfein, D., Huang, Y. Y., Chen, M., Bailey, C. H., and Kandel, E. R. (1998) Neuron 21, 813–825
10. Seeds, N. W., Verrall, S., Friedman, G., Hayden, S., Gadotti, D., Haffke, S., Christensen, K., Gardner, B., McGuire, P., and Krystosek, A. (1992) Ann.
