Tissue-Type Plasminogen Activator Crosses the Intact Blood-Brain Barrier by Low-Density Lipoprotein Receptor–Related Protein-Mediated Transcytosis

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Background—Accumulating evidence demonstrates a critical involvement of tissue-type plasminogen activator (tPA) in pathological and physiological brain conditions. Determining whether and how vascular tPA can cross the blood-brain barrier (BBB) to enter the brain is thus important, not only during stroke but also in physiological conditions.

Methods and Results—In the present work, we provide evidence in vivo that intravenous injection of tPA increases NMDA-induced striatal lesion in the absence of BBB leakage. Accordingly, we show that tPA crosses the BBB both after excitotoxic lesion and in control conditions. Indeed, vascular injected tPA can be detected within the brain parenchyma and in the cerebrospinal fluid. By using an in vitro model of BBB, we have confirmed that tPA can cross the intact BBB. Its passage was blocked at 4°C, was saturable, and was independent of its proteolytic activity. We have shown that tPA crosses the BBB by transcytosis, mediated by a member of the LDL receptor–related protein family.

Conclusions—We demonstrate that blood-derived tPA can reach the brain parenchyma without alteration of the BBB. The molecular mechanism of the passage of tPA from blood to brain described here could represent an interesting target to improve thrombolysis in stroke (Circulation. 2005;111:2241-2249.)

Key Words: plasminogen activators ■ stroke ■ brain ■ endothelium ■ thrombolysis

Tissue-type plasminogen activator (tPA) is one of 2 mammalian serine proteases that activate plasminogen into plasmin, which leads to fibrin digestion.1 This ability has been exploited to rescue blood perfusion after ischemic injury, and in 1996, the Food and Drug Administration approved tPA as a treatment of cerebral ischemia in humans; however, increasing evidence supports the idea that tPA could also potentiate stroke damage.2 Indeed, hippocampal neurons of tPA-deficient mice are resistant to excitotoxin-induced degeneration.3 Wang and colleagues4 have also reported that the ischemic lesion is reduced in mice with tPA deficiency. Moreover, intravenous injection of tPA has been shown to potentiate the ischemic lesion in tPA knockout mice4 and in some5 but not all6,7 studies performed on wild-type animals.

Three main hypotheses have been proposed to explain how tPA, within the parenchyma, exacerbates neuronal death after stroke. Indeed, tPA could mediate some of its actions either through plasmin, leading to the degradation of laminin,8 or through activation of microglia.9 More recently, we have shown that exogenous tPA could cleave the amino-terminal end of the NR1 subunit of the NMDA receptor, resulting in a potentiation of NMDA-induced Ca2+ influx and subsequent excitotoxic neuronal death.10,11

Although the deleterious effect of tPA produced in the cerebral parenchyma is well accepted, whether blood-derived tPA can penetrate the brain and thus also contribute to these detrimental processes remains to be established clearly. After intravenous injection, tPA has been detected by in situ zymography in brain slices of tPA knockout mice after cerebral ischemia.4 The simplest explanation could be that tPA invades the brain through a compromised BBB. Accordingly, the areas with tPA activity have been correlated to those with BBB breakdown assessed by MRI.12 However, in that study, tPA was injected tardily, 4 hours after the onset of ischemia,12 whereas in clinically relevant studies, tPA is often injected early after the insult, at a time when opening of the blood-brain barrier (BBB) has not occurred. Indeed, although the kinetics of BBB disruption after cerebral ischemia remain controversial, there is evidence that the integrity of the BBB is preserved when tPA is injected intravenously.13,14 Thus,
how could tPA reach the brain parenchyma to potentiate injury at times when there is no BBB breakdown?

Recently, it has been suggested that tPA per se is able to promote leakage of the BBB, which is in agreement with the ability of tPA to promote hemorrhages. This effect of tPA on BBB leakage appears to be mediated by the LDL receptor–related protein (LRP) and/or overactivation of the metalloprotease-9 (MMP-9). In contrast, other studies have not shown such BBB breakdown after tPA infusion, even in the acute stage of cerebral ischemia.

Thus, the major aim of the present study was to determine whether blood-derived tPA is able to reach the brain parenchyma and influence brain processes. First, we investigated whether vascular tPA alters neuronal outcome during an excitotoxic challenge. Then, we investigated whether blood-derived tPA can cross the intact BBB in vivo. Third, we used in vitro models of BBB to determine the mechanisms underlying the passage of tPA through the BBB.

Methods

All experiments were performed in the framework of the French legislation that governs animal experimentation.

Striatal Excitotoxic Lesions

Striatal lesions were performed in male Sprague Dawley rats (weight 280 to 320 g) under sevoflurane-induced anesthesia. Two microliters of NMDA (50 nmol) with or without recombinant plasminogen activator inhibitor-1 (rPAI-1; 2 µg) or tPA (3 µg) was injected into the striatum at coordinates 0.2 mm posterior, 3.5 mm lateral, and 5.5 mm ventral to the bregma. Five minutes after insertion of the needle, the solution was injected with a Hamilton syringe pump at a rate of 0.5 µL/min. The needle was removed 5 minutes later. tPA (1 and 10 mg/kg) or vehicle was injected intravenously over a 15-minute period. BBB was revealed on brain sections (20 µm thick) with the streptavidin-alexa555 (red), or in the second set of experiments, against glial fibrillary acidic protein (GFAP, red), and tPA (antibody raised against tPA, red), or in the third set of experiments, against glial fibrillary acidic protein (GFAP, red), collagen IV (blue), and biotin (streptavidin-alexa488).

Histological Analysis of Brain Lesion

After 24 hours, rats were killed, and the brains were removed and frozen in isopentane for histological analysis. Cryostat-cut coronal brain sections (20 µm) were stained with cresyl violet and analyzed with an image analyzer. For volume analysis, one section out of every 20 was stained and analyzed (for a total of 12 sections, which covered the entire lesion). Regions of interest were determined through the use of a stereotaxic atlas for the rat, and an image-analysis system (BIOCOM RAG 200) was used to measure the lesion, which corresponded to the nonstained area. Results are mean ± SD. Statistical tests included ANOVA with Bonferroni correction (n = 7 to 10 in each group).

Biotinylation of tPA and Injection for Detection in Brain

Biotinylated tPA was produced from pure recombinant human tPA (Actilyse) subjected to the protocol of biotinylation described by the manufacturer (Molecular Probes). Then, biotinylated tPA was separated by size-exclusion chromatography. Fractions of 0.5 mL were collected before immunoblotting against biotin. Then, fractions containing the highest immunoreactivities were pooled, and an aliquot was subjected to SDS-PAGE before silver staining, immunoblotting raised against biotin, and zymography assay. In the silver staining and immunoblotting, 2 bands were revealed. The upper band corresponded to the single-chain tPA with a molecular weight of ~69 kDa, and the lower band corresponded to the double-chain tPA with a molecular weight of ~38 kDa, as previously described by Bringmann and colleagues (data not shown). The same procedure was used for the biotinylation of albumin. Injections of biotinylated tPA were performed in male Swiss mice (25 to 30 g) under sevoflurane-induced anesthesia. Thirty minutes after excitotoxic lesion, intravenous injections of biotinylated tPA were performed in the tail vein. At the time indicated in the Figure legends, mice were perfused with heparinized physiological solution. Brains were fixed, and slices were revealed against biotin (streptavidin-alexa488, green) and tPA (antibody raised against tPA, red), or in the second set of experiments, against glial fibrillary acidic protein (GFAP, red), collagen IV (blue), and biotin (streptavidin-alexa488).

FITC Injection and Confocal Microscopy for Determination of BBB Permeability

FITC-dextran (77 kDa, green) and biotinylated tPA were injected intravenously. Two hours later, brains were harvested, fixed with paraformaldehyde, and cut in 60-µm-thick slices. Biotinylated tPA was revealed on brain slices with the streptavidin-alexa555 (red). Staining was then analyzed by 3D reconstruction with confocal microscopy. For confocal analyses, a Nikon Eclipse (TE2000-E) inverted microscope and Nikon 20x, 1.4 numerical aperture epifluorescence oil immersion objective were used. 3D reconstruction was performed with Nikon EZ-C1. Mechanically induced BBB breakdown for positive control was performed as described by Westergren and colleagues.

Cerebrospinal Fluid Sampling

Briefly, rats were anesthetized with halothane, and body temperature was maintained at 37°C by placing the rats on a heated blanket. The skin overlying the occipital bone was incised, and the underlying tissue was prepared so that the membrane between the occipital bone and the upper cervical vertebral was exposed. Cerebrospinal fluid (CSF; 30 µL) was aspirated by the introduction of a 29-gauge needle in the membrane.

SDS-PAGE Plasminogen-Casein Zymography

Zymography assay was performed by addition of plasminogen (4.5 µg/mL) and casein (1%) in 15% SDS polyacrylamide gel. Electrophoresis was performed at 4°C. Gels were washed with Triton X-100 (2.5%) and incubated for 2 hours at 37°C. Caseinolytic bands were visualized after Coomassie staining.

BBB Experiments

Briefly, bovine brain endothelial cells were isolated and characterized as described by Brillault and colleagues. After 3 weeks, coated filters were set in 6-well dishes that contained glial cells. Endothelial cells were plated on their upper side in 1.5 mL of medium at a concentration of 4 × 10⁵ cells/mL. Experiments were performed 5 days after confluence. tPA (20 µg/mL) was added in the luminal compartment (corresponding to the upper side; Figure 5a). Abluminal media were harvested at different times and analyzed by fluorogenic substrate (Spectrozyme, XF444, American Diagnostica) and zymography assay. For analysis of the effect of tPA on BBB permeability, tPA was added in the luminal face for 4 hours. Permeability of the BBB was then assessed by estimation of the passage of control molecules ([14C]sucrose and [3H]mulin).

Materials

Human recombinant tPA (Actilyse) was purchased from Boehringer Ingelheim, and rPAI-1 and tPA Stop [2,7-bis-(4-amidinobenzylidene)-cycloheptan-1-one dihydrochloride] were purchased from American Diagnostica. RAP (receptor associated protein; 39 kDa), an antagonist of LRP, was provided by Dr Guojun Bu. NMDA was from Tocris. The following specific primary antibodies were used: rabbit polyclonal anti-human claudin-1, rabbit polyclonal anti-human occludin, rabbit polyclonal anti-human zonula occludens-1 (ZO-1; Zymed Laboratories Inc), goat polyclonal anti-collagen IV (Southern Biotechnology Associates Inc), and rabbit anti-GFAP (Sigma Aldrich). Rabbit polyclonal anti-claudin-5 was provided by Dr Miki Furuse (Department of Cell Biology, Kyoto University of Medicine, Kyoto, Japan). The rabbit
Results

**tPA Potentiates Excitotoxic Lesions**

We first compared the effects of a parenchymal (intrastriatal) or intravenous injection of tPA in a model of excitotoxic injury induced by intrastriatal injection of NMDA in rats. Although intrastriatal injection of tPA (3 μg) alone failed to induce neuronal death, it potentiated the volume of the brain lesion induced by injection of NMDA (40%; Figure 1a). Interestingly, intravenous injection of tPA (1 mg/kg) also potentiated NMDA-induced lesions (Figure 1b). Similar effects were observed with 10 mg/kg of tPA (Figure 1b). In this latter condition, 2 of the 10 animals displayed brain hemorrhages near the needle tract and have been excluded from the analysis. These animals did not have a larger infarct, which suggests that microhemorrhages do not contribute to the deleterious effect of tPA. The same results were obtained in mice, in a model of intrastriatal injection of NMDA, with an intravenous injection of tPA at 1 mg/kg (data not shown). In addition, intrastriatal coinjection of PAI-1, a powerful inhibitor of tPA, with NMDA in rats decreased the deleterious effect of intravenous injection of tPA (Figure 1c). This suggests that the proteolytic activity of tPA in the striatum is directly involved in the neurotoxic action of intravenously injected tPA.

**Neither NMDA Injection nor Intravenous Infusion of tPA Affects BBB Permeability to 77-kDa Dextran**

To examine the integrity of the BBB, FITC-dextran (77 kDa) was injected intravenously in control and NMDA-injected animals, with or without an intravenous injection of tPA. Two hours later, brains were harvested, and leakage of the BBB was analyzed as the diffusion of FITC-dextran in the brain parenchyma. At this time, no residual activity of the injected tPA was detected in the corresponding plasma (Table).

FITC-dextran fluorescence was restricted to the lumen of the vessels without any sign of extravasation in all conditions tested (Figures 2a, 2b, 2c, and 2d). These results show that neither intravenous injection of tPA (Figures 2b and 2d) nor intrastriatal injection of NMDA (Figures 2c and 2d) alters the integrity of the BBB at least 2 hours after the injections. Similar results were obtained in the mouse model (data not shown). Figure 2e shows positive control of FITC-dextran extravasation at the site of the burr hole. Overall, these data show that blood-derived tPA must cross the intact BBB to potentiate striatal excitotoxic lesion.
which demonstrates that it corresponded to biotinylated tPA (Figure 3a). In addition, both stainings also were observed after NMDA injection. To confirm that these signals were the consequence of an extravasation of blood-derived tPA, biotinylated tPA was injected intravenously in the presence of FITC-dextran. Then, both FITC-dextran and biotinylated tPA were revealed from nonperfused brain sections. After 3D reconstruction of capillaries (5 to 7 μm) with confocal microscopy, we detected significant amounts of biotinylated tPA around capillaries (Figure 3c), with an extravasation of tPA up to 50 μm from the limits of the lumen (Figure 3d).

Moreover, double immunostaining was performed with antibodies against type IV collagen to reveal basal lamina and with streptavidin to reveal either biotinylated albumin or biotinylated tPA (Figure 4). The respective distribution of each fluorochrome was determined by an analysis of fluorescence intensity along a cross section of a capillary (Figures 4d, 4h, and 4l). After perfusion, immunoreactivity for biotinylated tPA was detected clearly outside the basal lamina as soon as 45 minutes after intravenous injection, which excludes the hypothesis of storage of the biotinylated tPA in endothelial cells (Figures 4i through 4n). Control experiments were performed with biotinylated albumin, because albumin displays a molecular weight close to that of tPA (66 versus 69 kDa, respectively) and because it is known to remain within the vasculature. As shown in Figures 4a through 4d, albumin failed to cross the intact BBB (Figures 4e through 4h). In contrast, after mechanically induced BBB breakdown,20 extravasation of albumin in the brain parenchyma was observed as a positive control (Figures 4i through 4j). In addition, Figures 4m and 4n, which include GFAP immunostaining, show that the tPA that crosses the intact BBB diffuses at a distance up to that of the astrocytic end feet.

We next estimated both plasmatic and CSF levels of tPA after its intravenous injection. In control conditions, as reported previously,25 tPA activity is higher in the CSF than in the plasma. We show that although plasmatic tPA activity was increased at 10 minutes after the end of the injection, it returned to basal levels after 1 hour (Table). At that time, an increase in tPA activity was detected in the CSF (4-fold increase, P<0.05), which supports the ability of tPA to cross the intact BBB in vivo.

tPA Crosses the BBB In Vivo

To ascertain whether blood-derived tPA crosses the intact BBB, we generated a biotinylated tPA, for its ability to be traced and differentiated from endogenous tPA. Biotinylated tPA was purified and its proteolytic activity tested with a plasminogen-casein zymography assay of tPA and biotinylated tPA was detected in the brain parenchyma of nonlesioned animals, with a localization all around the vessels (Figure 3a). Staining for biotin matched the immunoreactivities for tPA, which demonstrates that it corresponded to biotinylated tPA (Figure 3a). In addition, both stainings also were observed after NMDA injection. To confirm that these signals were the consequence of an extravasation of blood-derived tPA, biotinylated tPA was injected intravenously in the presence of FITC-dextran. Then, both FITC-dextran and biotinylated tPA were revealed from nonperfused brain sections. After 3D reconstruction of capillaries (5 to 7 μm) with confocal microscopy, we detected significant amounts of biotinylated tPA around capillaries (Figure 3c), with an extravasation of tPA up to 50 μm from the limits of the lumen (Figure 3d).

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tPA Crosses the BBB In Vitro

To investigate how tPA crosses the BBB, we used an in vitro model of BBB, which consists of a coculture of endothelial cells (harvested from brain capillaries) and glial cells (Figure 5a). Our model was previously characterized to closely mimic the in vivo situation,26 such as a high electrical resistance (up to 800 Ω/cm²), a very low permeability for sucrose and inulin, and constitutive tight junctions revealed by the presence of occludin, ZO-1, claudin-1, and claudin-5 immunostainings.21 For all of the in vitro experiments presented in the present study, a parallel measure of the passage of [14C]-sucrose, which is known to cross the BBB exclusively by a paracellular diffusion,21 was performed and revealed that none of the tested conditions induced a paracellular pathway (data not shown). The quantification of tPA activity in the abluminal compartment, both with zymography (Figure 5b) and with
fluorogenic assays (Figure 5c), showed that a significant amount of the tPA loaded in the upper compartment was found on the lower side of the BBB as early as 60 minutes after injection. In these conditions, endothelial cells do not release detectable amounts of tPA in the abluminal compartment (Figure 5b). Furthermore, these data show that tPA remains active after its passage and that 4% of the tPA present in the luminal compartment is able to cross the BBB within 2 hours (Figure 5c).

**tPA Does Not Alter the Structure of the BBB or Permeability to Sucrose or Inulin In Vitro**

To investigate whether tPA crosses the BBB by altering its integrity, we tested whether tPA (20 μg/mL) could affect the biophysical properties of the BBB in vitro. tPA alone (4 hours of incubation at 37°C) failed to influence basal permeability for either sucrose (Figure 6b) or inulin (data not shown). As a control, mannitol led to a dramatic increase in both sucrose and inulin permeabilities as indicators of BBB leakage. Similarly, immunostainings for ZO-1 (Figure 6a) and occludin (data not shown) were not affected by the presence of tPA compared with treatment with mannitol. Altogether, these data demonstrate that tPA does not influence the integrity of the BBB and confirm our in vivo observations that suggest that tPA is able to cross the intact BBB.

**tPA Crosses the BBB Independently of Its Proteolytic Activity**

Because tPA is a serine protease, we tested whether its proteolytic activity was necessary for its passage through the BBB. To address this question, we used a previously characterized inhibitor of tPA called tPA Stop and the biotinylated tPA to trace tPA independently of its proteolytic activity by revelation of the corresponding conditioned media with streptavidin-peroxidase. Control experiments were performed to confirm the inhibitory effect of tPA Stop against the proteolytic activity of tPA in the luminal compartment (Figure 7a). In the presence of tPA stop, biotinylated tPA was still detectable in the abluminal compartment, which suggests that tPA might be able to cross the BBB independently of its proteolytic activity (Figures 7b and 7c).

**Passage of tPA Through the BBB Is Temperature Dependent and Saturable**

To determine whether tPA crosses the BBB through a transendothelial pathway, the passage of tPA was performed at 4°C, a condition known to inhibit transcytosis. Although the paracellular pathway measured by the passage of sucrose was not affected by incubation of cells at 4°C (Pe=1.33±0.10 at 37°C versus 1.57±0.18 at 4°C, P>0.05), in this condition, the passage of tPA was reduced dramatically, which excludes a paracellular pathway for tPA (Figure 8). Moreover, the use of different doses of tPA showed that the passage of tPA is a saturable phenomenon (Figures 9a and 9b), which suggests the involvement of a receptor.

**LRP Mediates the Passage of tPA Through the Intact BBB**

Because 2 main receptors have been involved in the internalization of tPA, LRP and the mannose receptor, we tested their respective contributions in the passage of tPA across the BBB. Although an excess of mannose (100 mmol/L) failed to

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**Figure 4. Blood-derived tPA crosses intact BBB.** Biotinylated tPA (i through n) or biotinylated albumin (a through h) was injected intravenously in control condition (a through d and i through n) or in mechanically induced BBB disruption (e through h). Forty-five minutes later, animals were perfused, brains were harvested and cut in 60-μm-thick slices and biotinylated proteins were revealed on brain slices with streptavidin-alexa488 (green; b, f, and j). Regular immunostaining with antibody raised against collagen type IV (blue; a, e, and i) and GFAP (red; m) was performed on same brain sections. c, g, and k are superimpositions of immunostainings against biotin and collagen IV. d, h, and l correspond to intensity of fluorescence for each pixel present in line crossing capillary as indicated in c, g, and k. n Superimposition of immunostainings against biotinylated tPA, collagen IV, and GFAP of i through k. Scale bar indicated in a and e =25 μm. Scale bar in i =5 μm. biot indicates biotinylated.
influence the passage of tPA through the BBB, the LRP antagonist RAP (39 kDa; 500 nmol/L) blocked the passage of tPA (Figures 10a and 10b). RAP previously has been shown to inhibit the interaction between LRP and tPA at 200 and 500 nmol/L.24 Here, the ability of RAP to prevent the binding of iodinated tPA to LRP was addressed in MH1C1 cells. In this model, RAP at 200 nmol/L prevented 75% of the binding of tPA to LRP (data not shown). Altogether, these data suggest that the passage of tPA through the intact BBB is an LRP-mediated transcytosis.

Discussion

Although tPA is currently used in clinical practice for thrombolysis in peripheral or cerebral ischemia, the balance between its beneficial thrombolytic activity and its side effects is a matter of debate. This is especially the case for stroke, for which conflicting results have been obtained after intravenous injection of tPA on several models of cerebral ischemia or excitotoxic lesions in animals.2 It has been hypothesized that tPA is beneficial in the vascular compartment essentially because of reperfusion of the ischemic tissue, but that it is deleterious in the brain parenchyma. To identify and detail the mechanism of the harmful effect of tPA in stroke, several authors have used a mechanical model of ischemia in which it was supposed that tPA should not have a beneficial action, because in these conditions, reperfusion will occur independently of the thrombolytic action of tPA.7 Surprisingly, intravenous injection of tPA in such stroke models still has a beneficial component through the degradation of endogenous deposits of fibrin that takes place in the ischemic tissue.27,28 Another key point of the debate is the dose of tPA used to achieve reperfusion. Because human tPA (found in Actilyse) is 10-fold less efficient in activating rodent plasminogen than endogenous rodent tPA, the dose used in animals is usually 10 mg/kg, corresponding to 10-fold the dose used in humans, which leads to a complex interpretation of the data. That is why in the present study, we attempted to further investigate the real influence of blood-derived tPA in the evolution of the excitotoxic lesion. Indeed, excitotoxic neuronal injury is the main process in the pathogenesis of an ischemic lesion, and tPA has been shown to potentiate the excitotoxic injury both in vivo and in vitro.2 Using the intrastriatal injection of NMDA-induced injury, we have shown that tPA potentiates the excitotoxic lesion both when coinjected with NMDA in the striatum and when injected intravenously. Moreover, the effect of tPA is prevented by an intrastriatal injection of the tPA inhibitor PAI-1, which confirms the deleterious effect of the proteolytic activity of tPA in the brain parenchyma. Altogether, these results also suggest that blood-derived tPA is able to cross the BBB.

The next step was to determine whether tPA is able to cross the BBB in vivo. In all the protocols used in this part of the study, tPA was injected for 15 minutes. The half-life of tPA in plasma is very short, ranging from 1 to 4 minutes in rodents and from 5 to 10 minutes in humans.29 In a previous study, Godfrey and colleagues30 showed that plasmatic tPA activity was no longer detected 2 hours after a 30-minute intravenous infusion (1 mg/kg). In the present study, tPA was no longer detectable 1 hour after the injection. Thus, our first control was to check whether there was a leakage in the BBB during the 2 hours after tPA infusion with or without intrastriatal
injection of NMDA. Because no BBB leakage was observed during the time of the experiment, the observation of an extravasation of the biotinylated tPA from the vessel to the brain parenchyma confirms the ability of blood-derived tPA to reach the parenchyma even in the absence of BBB disruption. Although our immunohistochemical approach is not quantitative, we demonstrated that the detectable amount of blood-derived tPA that crosses the BBB in vivo is sufficient to lead to a 4-fold increase in bioactive tPA in the CSF at 1 hour and to potentiate striatal excitotoxic lesion. These findings have important implications. Indeed, although several studies have documented an increased permeability of the BBB after brain injuries, it has also been shown that after cerebral ischemia, an immediate and complete BBB breakdown cannot be observed but these changes occur over time.13,14 Here, we show that tPA is able to cross the BBB without the requirement of its breakdown. Indeed, we show that tPA crosses the intact BBB independently of its proteolytic activity and that this process is mediated through a receptor-dependent mechanism identified as a member of the LRP receptor family. These 2 observations are consistent, because tPA is known to bind LRP through its EGF domain independently of its catalytic activity31 and because LRP has been shown both in vitro32 and in vivo33 to be a high-capacity
transport system that mediates transcytosis of protein (such as lactoferrin or RAP) from blood to brain. In the present in vitro studies, we used 20 μg/mL tPA, given the fact that although the concentration of blood tPA is 1–10 ng/mL in physiological conditions, after thrombolysis in rats (tPA at 10 mg/kg), it reaches 30 μg/mL after a 30-minute injection. tPA has no effect on the integrity of the BBB in physiological conditions either in vitro or in vivo. Some authors but not all suggest that tPA amplifies ischemia-induced BBB breakdown. Interestingly, the studies that showed an effect of tPA on BBB were performed on hypertensive rats. In a recent report, Yepes and colleagues showed that tPA can lead to an opening of the BBB by a mechanism that involves LRP. This discrepancy with the present results could be explained by the fact that here, tPA was injected intravenously, as it is usually performed in humans, whereas it was injected directly into the CSF in the study published by Yepes and colleagues. In addition, although neuronal death is prevented in tPA-deficient mice, Chen and colleagues have shown that excitotoxic injury leads to a late BBB breakdown (24 hours after the injection of kainate) in both wild-type and knockout animals, which suggests that tPA is not necessary for BBB disruption and that the establishment of the lesion and the BBB breakdown are independent processes.

Overall, the present data provide evidence that tPA does not affect the integrity of the BBB but that it can reach the brain parenchyma, which suggests that blocking the passage with RAP or using a modified tPA to prevent its interaction with LRP could be a new strategy to improve thrombolytic therapy. Because LRP is one of the main receptors involved in the clearance of tPA, its blockade would have 2 interesting effects: first, an increased plasmatic half-life of tPA, as already shown, and at the same time, a decreased level of tPA in the brain parenchyma. Altogether, the present data confirm the deleterious effect of blood-derived tPA against excitotoxic lesions and show that blood-derived tPA can cross the intact BBB.

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